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**Prevalence of Vitamin D Deficiency in Pregnant  
Women and its Association with Gestational  
Diabetes Mellitus**

**By**

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A thesis submitted to  
The Faculty of Medicine  
of the University of Warwick  
for the degree of

**DOCTOR OF PHILOSOPHY**

Diabetes & Metabolism  
Clinical Sciences Research Laboratories  
Warwick Medical School  
University of Warwick  
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## **DECLARATION**

I declare that this thesis is an accurate record of my results, obtained by myself working with the research teams at Warwick Medical School, University of Warwick and associated labs in the Department of Biochemistry at the College of Science in King Saud University (KSU). The data that have arisen are detailed in this thesis. All sources of support and technical assistance have been stated in the text of the acknowledgements. None of the work has been previously submitted for a higher degree.

All sources have been specifically acknowledged using references.

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## **DEDICATION**

This thesis is dedicated to my family, who provided me with so much support, love and guidance throughout.

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## SYNOPSIS

Several reports have linked vitamin D deficiency with an increased risk of gestational diabetes mellitus (GDM). Both of these conditions are alarmingly common in Saudi Arabia, and pose additional risk of developing future metabolic disease. This study, therefore, investigates the vitamin D status amongst pregnant Saudi women, and the potential influence of vitamin D deficiency on metabolic dysfunction, such as GDM.

A total of 578 pregnant women ( $28.8 \pm 5.4$  years) were recruited for this study during their first trimester of pregnancy (8-12 weeks) and followed up in their second trimester (24-28 weeks), where data were collected from 297 [51.3% (297/578);  $28.9 \pm 5.3$  years] women. The study collected socio-economic, anthropometric and biochemical data, along with dietary intake, physical activity and sun indices.

The findings of this study indicate that during the first trimester 81% of women being vitamin D deficient, dropping to 77% in the second trimester. It was also noted that being younger in age, multiparous, having a lower level of education, being a housewife, and living in West Riyadh were all associated with vitamin D deficiency during the first trimester ( $p < 0.05$ ), and this further corresponded to reduced sun exposure ( $p < 0.001$ ). In contrast, physically active pregnant women, women adequately exposed to sunlight at noon ( $p < 0.001$ ), and residents of North Riyadh all had significantly higher circulating vitamin D levels ( $p < 0.05$ ). Furthermore, low levels of high-density lipoprotein cholesterol (HDL-cholesterol) during early pregnancy were also associated with an increased risk of vitamin D deficiency ( $p < 0.05$ ). Ultimately, compared with the first trimester, circulating vitamin D levels were significantly higher in the second trimester, after adjustment ( $p < 0.001$ ).

Among the pregnant women studied here, it was subsequently found that 33% developed GDM in the second trimester. Vitamin D deficiency in early pregnancy was associated with significantly higher risk of GDM, and this risk persisted after adjusting for confounding risk factors with regard to both vitamin D deficiency and GDM [odds ratio (OR) 3.97, confidence interval (CI) 1.12-14.15,  $p = 0.033$ ]. In addition,



significantly higher random blood glucose levels, higher glycated haemoglobin (HbA1c), and low HDL-cholesterol in early pregnancy were observed in the GDM subjects, compared to those without GDM ( $p < 0.05$ ). Furthermore, vitamin D deficiency in mid-pregnancy increased the risk of metabolic syndrome and low HDL-cholesterol, thus pointing to the role of vitamin D in the probability of developing cardiometabolic disease.

In summary, a high prevalence of vitamin D deficiency was observed amongst the subjects in this study, namely pregnant Saudi women. Moreover, hypovitaminosis D in early pregnancy was identified as a significant risk factor for the development of GDM. The present study, therefore, suggests that maintaining optimal levels of vitamin D during pregnancy may be a useful intervention in preventing the development of GDM and metabolic syndrome. Along with vitamin D supplementation, lifestyle modification also appears to be critical for maintaining optimal vitamin D levels during pregnancy, thus avoiding pregnancy-related complications.

## ABBREVIATIONS

<b>ACOG</b>	American College of Obstetricians and Gynaecologists
<b>ADA</b>	American Diabetes Association
<b>ANCOVA</b>	Analysis of covariance
<b>ANOVA</b>	Analysis of variance
<b>AODM</b>	Adult Onset diabetes mellitus
<b>BAP</b>	Bone-specific alkaline phosphatase
<b>β-cell</b>	Beta cells
<b>BMI</b>	Body mass index
<b>BRP</b>	Biomarkers Research Program
<b>BP</b>	Blood pressure
<b>Calc. intake</b>	Calcium intake
<b>CHD</b>	Coronary heart disease
<b>CI</b>	Confidence interval
<b>CMACE</b>	Centre for Maternal and Child Enquiries
<b>CONSORT</b>	Consolidated standards for the reporting of trials
<b>CRP</b>	C-reactive protein
<b>CV</b>	Coefficients of variation
<b>CYP24A1</b>	1,25(OH) <sub>2</sub> D <sub>3</sub> -24-hydroxylase
<b>CYP27B1</b>	25(OH)D-1-α-hydroxylase
<b>CYP2R1</b>	Enzyme 25-hydroxylase
<b>DEQAS</b>	Vitamin D External Quality Assessment Scheme
<b>DRI</b>	Dietary reference intake
<b>ECLA</b>	Electrochemiluminescence assay
<b>ECLIA</b>	Electrochemiluminescence binding assay
<b>EDD</b>	Estimated date of delivery
<b>EDTA</b>	Ethylene diamine tetra-acetic acid
<b>FEV<sub>1</sub></b>	Forced expiratory volume in 1
<b>FFQ</b>	Food frequency questionnaire
<b>GDM</b>	Gestational diabetes mellitus
<b>GWG</b>	Gestational weight gain
<b>HAART</b>	Highly active antiretroviral therapy
<b>HAPO</b>	Hyperglycaemia and adverse pregnancy outcome
<b>HbA1c</b>	Haemoglobin A1c
<b>HBP</b>	High blood pressure
<b>HDL- cholesterol</b>	High density lipoprotein cholesterol
<b>HOMA-IR</b>	Homoeostasis Model Assessment of Insulin Resistance
<b>HOMA-β</b>	Homoeostasis Model Assessment of Beta Cells
<b>HTN</b>	Hypertension
<b>IADPSG</b>	International Association of Diabetes and Pregnancy Study Groups
<b>IDF</b>	International Diabetes Federation
<b>IFN-γ</b>	Interferon-γ
<b>IGT</b>	Impaired glucose tolerance
<b>IL-6</b>	Interlukin-6
<b>IOM</b>	Institute of Medicine
<b>IPAQ</b>	International Physical Activity Questionnaire

<b>IU</b>	International unit
<b>gm</b>	Grams
<b>KFMC</b>	King Fahad Medical City
<b>Kg</b>	Kilograms
<b>KKUH</b>	King Khalid University Hospital
<b>KSA</b>	Kingdom of Saudi Arabia
<b>KSU</b>	King Saud University
<b>LDL-cholesterol</b>	Low-density lipoprotein cholesterol
<b>LC-MS/MS</b>	Liquid chromatography–mass spectrometry
<b>LMP</b>	Last menstrual period
<b>MAC</b>	Mid-upper arm circumference
<b>mins</b>	Minutes
<b>MS</b>	Multiple sclerosis
<b>NHLBI/AHA</b>	National Heart, Lung and Blood Institute/American Heart Association
<b>nm</b>	Nanometer
<b>NPST</b>	National Plan for Science and Technology
<b>NZSSD</b>	New Zealand Society for the Study of Diabetes
<b>OGTT</b>	Oral glucose tolerance test
<b>OR</b>	Odds ratio
<b>oz</b>	Ounce
<b>PA</b>	Physical activity
<b>PCOS</b>	Polycystic ovarian syndrome
<b>POC</b>	Point-of-care
<b>PPWR</b>	Post-partum weight retention
<b>PTH</b>	Parathyroid hormone
<b>PTHrP</b>	Parathyroid hormone-related protein
<b>RA</b>	Rheumatoid arthritis
<b>RCT</b>	Randomised controlled trials
<b>RDA</b>	Recommended daily allowance
<b>SFT</b>	Skin fold thickness
<b>SOS</b>	Saudi Osteoporosis Society
<b>T1DM</b>	Type 1 diabetes mellitus
<b>T2DM</b>	Type 2 diabetes mellitus
<b>TG</b>	Triglycerides
<b>TNF-<math>\alpha</math></b>	Tumour necrosis factor- $\alpha$
<b>TB</b>	Tuberculosis
<b>UAE</b>	United Arab Emirates
<b>UK</b>	United Kingdom
<b>URI</b>	Urinary tract infection
<b>USA</b>	United States of America
<b>USD</b>	United States dollar
<b>USDA</b>	U.S. Department of Agriculture
<b>UVB</b>	Ultraviolet-B
<b>V1</b>	Visit 1
<b>V2</b>	Visit 2
<b>VDBP</b>	Vitamin D-binding protein

<b>VDR</b>	Vitamin D receptor
<b>VLDL</b>	Very low-density lipoprotein
<b>Vs</b>	Versus
<b>WHO</b>	World Health Organization
<b>WHR</b>	Waist-to-hip ratio
<b>wk</b>	Week
<b>1,25(OH)<sub>2</sub>D</b>	1,25-hydroxyvitamin D
<b>25(OH)D</b>	25-hydroxyvitamin D
<b>Δ Body fat</b>	Change in body fat

# **Chapter 1**

## **Introduction**

## **1.1 Vitamin D During Pregnancy**

### **1.1.1 Background**

Vitamin D was recognised as a fat-soluble vitamin, and identified as a calciferol in the early 20th century (Ross et al., 2011). Vitamin D exists in two main forms: vitamin D<sub>2</sub> (ergocalciferol) and vitamin D<sub>3</sub> (cholecalciferol) (Holick, 2006a). Although people can synthesise some vitamin D in their bodies, the amount may not be sufficient due to a variety of factors, meaning that additional sources may be required to maintain adequate levels. Vitamin D is in fact classed as a hormone, rather than a vitamin, due to the occurrence of specific receptors (Holick, 2011b). Nearly all cells in the human body contain vitamin D receptors (VDRs), implying that they require vitamin D to function appropriately (Holick, 2011a; Holick, 2011b).

It is apparent that sufficient vitamin D levels are essential for maintaining other functions in the human body, including the brain, heart muscle, and adipose tissue (Bikle, 2009). In addition, vitamin D controls certain genes that regulate metabolic control, immune function, and cell growth and development (Holick, 2011b; Wacker & Holick, 2013b). Research has demonstrated that hypovitaminosis D is linked to several chronic disorders, including cancer, infections, autoimmune diseases, neurological diseases, cardiovascular diseases, and type 2 diabetes mellitus (T2DM) (Holick, 2011a; Holick, 2011b). These findings have led to an investigation into the novel functions of vitamin D in various human cells, and shed light on its implications for health and disease.

#### ***1.1.1.1 Sources of Vitamin D***

Vitamin D may be acquired through endogenous mechanisms, such as skin-based sun exposure, or exogenously from a diet of food naturally rich in vitamin D, or fortified food and supplements (Alpert & Shaikh, 2007). For example, vitamin D<sub>3</sub> can be synthesised by the epidermis in response to ultraviolet-B (UVB) radiation from the sun, or obtained from the diet. Whereas vitamin D<sub>2</sub> is converted from ergosterol, which is synthesised in plants exposed to ultraviolet radiation (DeLuca & Zierold, 1998). The

production of vitamin D<sub>3</sub> from 7-dehydrocholesterol in the epidermis occurs because of stimulation from UVB of wavelength 290-315 nm (Norman, 2001). Hence, the endogenous production of vitamin D based on UVB radiation depends on the amount of UVB reaching the skin, and the presence of 7-dehydrocholesterol (Holick, 1995a).

Dietary sources constitute less than 10% of daily vitamin D intake, due to the limited sources of vitamin D available in food (Holick et al., 2012). According to the Institute of Medicine (IOM), foods naturally rich in vitamin D include salmon, oily fish, and extracted cod liver oil (IOM, 2010), with lesser concentrations being found in egg yolk and sun-dried mushrooms (Ovesen et al., 2003; Holick, 2011a). Although fortified foods, such as dairy products, cereals, and juices are good sources of vitamin D (IOM, 2010), in Saudi Arabia, few foods are fortified with vitamin D. Under the current regulations, regularly consumed food is not fortified, but even when it is, it is to a lesser degree than is recommended by the United States of America (USA) market guidelines (Sadat-Ali et al., 2013). Vitamin D-fortified foods in Saudi Arabia include milk, which provides a range from 40-400 international units (IU) and enriched wheat and enriched treated flour (55.1 IU/100 gm) (Sadat-Ali et al., 2013).

Conversely, in the USA and Canada, milk is voluntarily supplemented with approximately 100-400 IU/cup (IOM, 2010), although cheese, ice-cream, and other dairy products are not usually fortified. Additionally, cereals, orange juice, yoghurt, margarine, and other nutritional products may be fortified with vitamin D (IOM, 2010). Supplementation with over-the-counter medication forms another important dietary source of vitamin D (IOM, 2010). A list of vitamin D sources is presented in the following Table (**Table 1.1**).

**Table 1.1 Vitamin D sources, adapted from Holick (2007) and Holick et al. (2011a).**

<b>Natural Sources</b>	
Cod liver oil	~400–1,000 IU/teaspoon vitamin D <sub>3</sub>
Salmon, fresh wild and caught	~600–1,000 IU/3.5 oz. vitamin D <sub>3</sub>
Salmon, fresh farmed	~100–250 IU/3.5 oz. vitamin D <sub>3</sub> , vitamin D <sub>2</sub>
Salmon, canned	~300–600 IU/3.5 oz. vitamin D <sub>3</sub>
Sardines, canned	~300 IU/3.5 oz. vitamin D <sub>3</sub>
Mackerel, canned	~250 IU/3.5 oz. vitamin D <sub>3</sub>
Tuna, canned	236 IU/3.5 oz. vitamin D <sub>3</sub>
Shiitake mushrooms, fresh	~100 IU/3.5 oz. vitamin D <sub>2</sub>
Shiitake mushrooms, sun-dried	~1,600 IU/3.5 oz. vitamin D <sub>2</sub>
Egg yolk	~20 IU/yolk vitamin D <sub>3</sub> or D <sub>2</sub>
Sunlight/UVB radiation	~20,000 IU, equivalent to exposure to one minimal erythral dose in a bathing suit. Thus, exposure of arms and legs to a 0.5 minimal erythral dose is equivalent to ingesting ~3,000 IU vitamin D <sub>3</sub> .
<b>Fortified Foods</b>	
Fortified milk	100 IU/8 oz., usually vitamin D <sub>3</sub>
Fortified orange juice	100 IU/8 oz. vitamin D <sub>3</sub>
Infant formulas	100 IU/8 oz. vitamin D <sub>3</sub>
Fortified yogurts	100 IU/8 oz., usually vitamin D <sub>3</sub>
Fortified butter	56 IU/3.5 oz., usually vitamin D <sub>3</sub>
Fortified margarine	429 IU/3.5 oz., usually vitamin D <sub>3</sub>
Fortified cheeses	100 IU/3 oz., usually vitamin D <sub>3</sub>
Fortified breakfast cereals	~100 IU/serving, usually vitamin D <sub>3</sub>
<b>Pharmaceutical Sources in the US</b>	
Vitamin D <sub>2</sub> (ergocalciferol)	50,000 IU/capsule
Drisdol (vitamin D <sub>2</sub> ) liquid	8,000 IU/cc
<b>Supplementary Sources</b>	
Multivitamins	400, 500, 1,000 IU vitamin D <sub>3</sub> or vitamin D <sub>2</sub>
Vitamin D <sub>3</sub>	400, 800, 1,000, 2,000, 5,000, 10,000, and 50,000 IU
<b>Note:</b> IU = 25 ng; oz. = ounce	



### ***1.1.1.2 Metabolism and Absorption of Vitamin D***

Vitamin D, which is either synthesised in the body or consumed from food, is biologically inactive. It binds to a vitamin D binding protein (VDBP), or lipoproteins in the blood, and is transported to the liver (Lips, 2006; IOM, 2010) where the enzyme, 25-hydroxylase (CYP2R1) hydroxylates it to 25(OH)D (calcidiol). This undergoes further hydroxylation in the kidneys, by the enzyme 25(OH)D-1- $\alpha$ -hydroxylase (CYP27B1), to yield 1,25-hydroxyvitamin D [1,25(OH)<sub>2</sub>D] (calcitriol). 1,25(OH)<sub>2</sub>D is the active form, and is primarily produced in the kidneys, but may also be synthesised in other tissue, such as the pancreas, immune system, and placenta (Holick, 2006b; Wacker & Holick, 2013a; Bikle, 2014). The enzyme responsible for catabolising 1,25(OH)<sub>2</sub>D to its less active form and water-soluble metabolites is 1,25(OH)<sub>2</sub>D<sub>3</sub>-24-hydroxylase (CYP24A1), which is upregulated by 1,25(OH)<sub>2</sub>D itself, in a negative feedback mechanism (Wacker & Holick, 2013a; Bikle, 2014).

Renal CYP27B1 stimulators include, higher concentrations of parathyroid hormone (PTH), hypocalcaemia, hypophosphatemia, and calcitonin, whereas inhibitors of this enzyme include fibroblast growth factor-23, hyperphosphatemia, and 1,25(OH)<sub>2</sub>D itself (Kawashima et al., 1981; Nesbitt & Drezner, 1993; Holick, 2007). Additionally, pregnancy, lactation, prolactin, sex steroids, growth spurt, growth hormone, and insulin-like growth factor 1, all play a part in improving the induction of renal CYP27B1, and therefore the production of 1,25(OH)<sub>2</sub>D, to meet higher calcium needs (Bouillon, 2001; Holick, 2004). However, non-classical CYP27B1 expression in other bodily tissues is tissue-specific (Olmos-Ortiz et al., 2015). For example, studies have shown that monocyte and macrophage derived CYP27B1 is stimulated by tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ), but not PTH (Pryke et al., 1990; Gyetko et al., 1993). In a comparable manner, pro-inflammatory cytokines stimulate CYP27B1 and CYP24A1 production in the placenta for synthesis and inactivation of vitamin D, respectively (Noyola-Martínez et al., 2014). Hence, it is interesting to note that both the anabolism and catabolism of placental 1,25(OH)<sub>2</sub>D are locally affected by inflammatory cytokines (Noyola-Martínez et al., 2014).

In the small intestine, dietary vitamin D (either D<sub>2</sub> or D<sub>3</sub>) is absorbed with other foods containing fat, due to its fat-soluble nature. Therefore, its efficiency is based on the fat in the gut lumen (Holick, 1995a). When there is sufficient vitamin D, 30% of dietary calcium is absorbed, whereas only 10-15% of dietary calcium is absorbed from the small intestine if no vitamin D is present (Heaney et al., 2003). During pregnancy, lactation, and growth, calcium absorption can increase by up to 80%, due to the higher calcium demand (Holick, 2004).

After its absorption, vitamin D is incorporated into chylomicrons before reaching the systemic circulation via the lymphatic system, and is finally transported to the liver and kidneys, or non-classical tissues (Ross et al., 2011). Excess vitamin D is primarily taken up by adipose tissue and skeletal muscle (Jones, 2008).

#### ***1.1.1.3 Functions of Vitamin D***

Vitamin D is vital for several biological actions of the human body. For example, 1,25(OH)<sub>2</sub>D binds to VDRs facilitating the control of transcriptional activity, which is cell-specific (John et al., 2007; Bikle, 2009). VDRs are extensively distributed throughout the body's tissues, indicating there are many different biological actions of vitamin D (John et al., 2007), some considered classical, and others non-classical. Classical actions involve the expression of VDR in the intestine, bones, and kidneys, along with the synthesis of parathyroid and thyroid hormones, calcium regulation, and bone metabolism (Deluca & Cantorna, 2001; Holick, 2002, 2004; IOM, 2010). Non-classical functions involve the presence of VDRs in other cells, including the pancreas, stomach, brain, heart, gonads, activated T and B lymphocytes, skin, and placenta, which pertains to the production of active vitamin D (Stumpf et al., 2007; Bikle, 2009). Hence, 1,25(OH)<sub>2</sub>D encourages the induction of insulin secretion, apoptosis, inhibition of cell proliferation, angiogenesis, and renin production, all of which are also non-classical functions of vitamin D (Holick, 2010; Hossein-Nezhad & Holick, 2013).

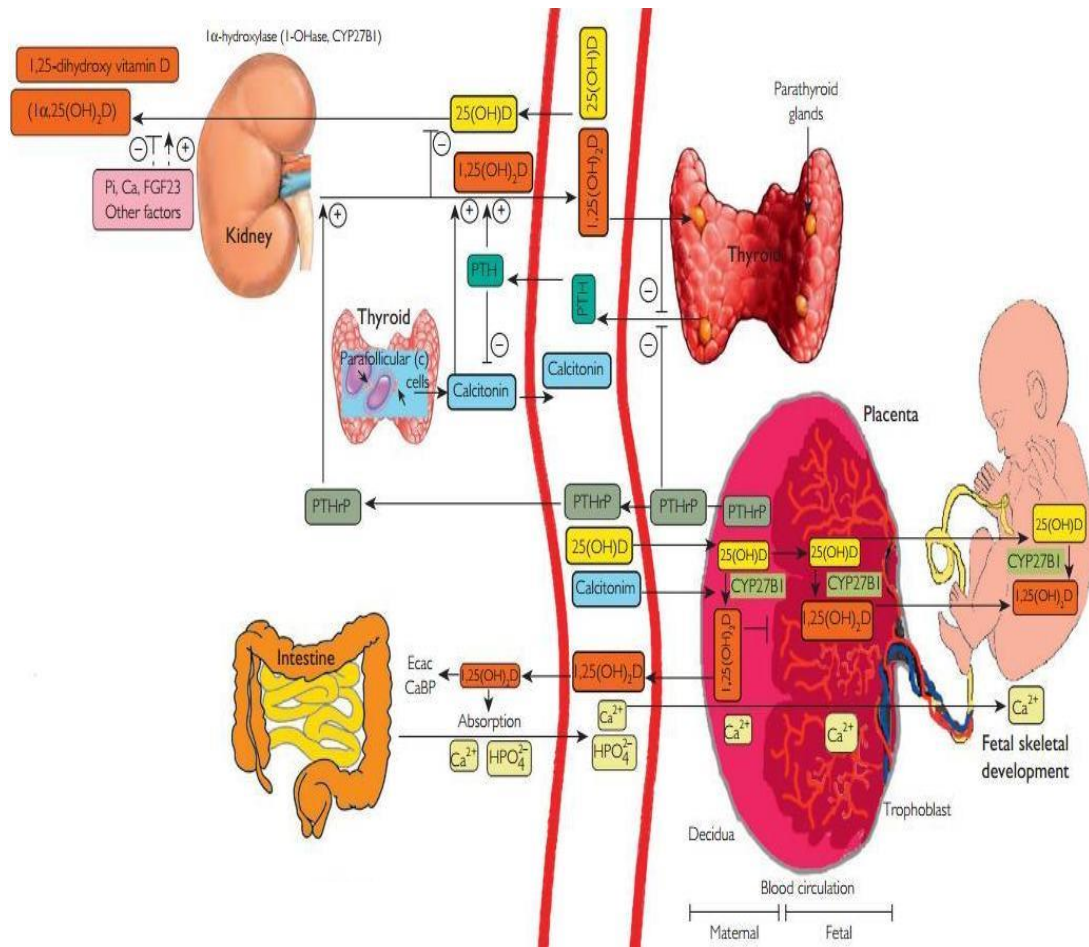
Vitamin D has other functions, including the reduction of inflammation, and modulation of neuromuscular and immune function (Hayes et al., 2003; Holick, 2003b).

Moreover, it has a critical role in the immune system, by inhibiting adaptive immunity and promoting innate immunity (Bikle, 2009). These extensive activities of 1,25(OH)<sub>2</sub>D have been further hypothesised as having a possible role in the prevention of cancer or development of cancer therapies (Masuda et al., 2005), long with the treatment of other chronic conditions, a such as auto-immune conditions, cardiovascular disease, and infections (Holick, 2008).

#### ***1.1.1.4 Homoeostasis of Vitamin D During Pregnancy***

During gestation, the body undergoes numerous changes intended to optimise the nutrition and health of the offspring. However, the behaviour of vitamin D during gestation is not yet fully understood (IOM, 2010). For example, the conversion of vitamin D to 25(OH)D during gestation it not what makes it unique; it is the conversion of 25(OH)D to 1,25(OH)<sub>2</sub>D where the dissimilarities appear. This is due to the location of the VDRs and vitamin D-activating enzyme in maternal (decidual) and foetal (trophoblastic) components of the placenta, which can synthesise the active form of vitamin D (Evans et al., 2004; Shin et al., 2010). By contrast, the vitamin D catabolic enzyme is poorly expressed in the placenta during early pregnancy, which coincides with the body's need to preserve active vitamin D, because of the increased bone growth demands of the foetus, and the needs of the mother (Evans et al., 2004).

Vitamin D in the form of 25(OH)D can readily pass from the placenta to the kidneys of the foetus, whereas, 1,25(OH)<sub>2</sub>D is incapable of passing through the placenta (Thandrayen & Pettifor, 2010). Accordingly, the foetus is wholly dependent on 25(OH)D placental delivery, so the mother's vitamin D metabolism undergoes a series of adaptations after the maternal intake and production of 25(OH)D (Kovacs, 2008). Thus, levels of 1,25(OH)<sub>2</sub>D in pregnant women increase in both the kidneys and placenta (Lapillonne, 2010) (**Figure 1.1**).



**Figure 1.1** Vitamin D metabolism during pregnancy. 25(OH)D is thought to easily cross the maternal placenta. The placenta expresses VDRs and produces 25(OH)D-1- $\alpha$ -hydroxylase (CYP27B1) to convert 25(OH)D to 1,25(OH)<sub>2</sub>D. However, 1,25(OH)<sub>2</sub>D does not readily cross the placenta, and foetal 1,25(OH)<sub>2</sub>D levels are normally lower than maternal serum levels. The low foetal concentrations of 1,25(OH)<sub>2</sub>D also reflect low foetal parathyroid hormone (PTH) and high phosphorus concentrations, which suppress renal CYP27B1. Although parathyroid hormone-related protein (PTHrP) is elevated in the foetal circulation, it appears to be less capable than PTH to stimulate renal CYP27B1 (adapted from Hossein-Nezhad & Holick, 2013).

It is noticeable that maternal serum concentrations of 1,25(OH)<sub>2</sub>D gradually increase during gestation, with an increase of 50-100% in the first and second trimester, and 100% by the end of pregnancy (Ritchie et al., 1998; Christesen et al., 2012) (**Figure 1.1**). However, the reasons for this increase in 1,25(OH)<sub>2</sub>D remain unclear. It may be due to the elevated production of renal CYP27B1 in pregnancy from the mother and foetus (Hosseini-Nezhad & Holick, 2013), along with extra production from the placenta and decidua (Shin et al., 2010; Barrett & McElduff, 2010). However, given an increase in 1,25(OH)<sub>2</sub>D, expectant mothers are likely to have higher cellular exposure to vitamin D during the second half of gestation, suggesting a function of vitamin D in obstetric well-being.

Regarding serum 25(OH)D levels, it is unclear whether any changes occur during pregnancy under normal conditions (Ginde et al., 2010). During pregnancy, the body attempts to compensate its levels to achieve sufficiency, by reducing the renal clearance of 25(OH)D. However, due to an elevated plasma volume during pregnancy, haemodilution is induced, so levels of vitamin D may be subsequently counterbalanced (Marwaha et al., 2011; McAree et al., 2013). Furthermore, 25(OH)D levels may vary due to environmental or behavioural confounding factors (Bodnar et al., 2007b). Thus, both endogenous and exogenous factors during pregnancy can affect vitamin D levels. The understanding of 25(OH)D concentration is inconclusive so far, as some studies state that 25(OH)D concentrations significantly increase or decrease during pregnancy (Zhang et al., 2014; Choi et al., 2015), while others indicate that there is no change (Marwaha et al., 2011). It is important to note here that these changes in vitamin D levels are not connected to changes in calcium absorption within the intestine (Ross et al., 2011). Thus, the increased calcium absorption that occurs during pregnancy is independent of 1,25(OH)<sub>2</sub>D, because calcium is stimulated by maternal hormones, such as prolactin and placental lactogen (Kovacs, 2008).

In non-pregnancy, studies have shown that PTH is strongly inversely related to vitamin D status, especially in the context of suboptimal 25(OH)D status (Kuchuk et al., 2009; Sai et al., 2010). The response of PTH levels to gestation show varied and inconsistent results, with a pattern of unchanged levels in early pregnancy and higher levels towards

the time of delivery (Kovacs & Kronenberg, 1997; Essley et al., 2012). The reaction of parathyroid hormone to pregnancy may be altered by variations in 25(OH)D concentration, regularity of calcium intake, and the age of the woman in pregnancy (Bezerra et al., 2002; Haddow et al., 2011). A cross-sectional study conducted on women in early gestation, showed that parathyroid hormone in African Americans had a weak ( $r = -0.074$ ) inverse correlation with vitamin D levels (Haddow et al., 2011). Another study conducted on women in mid- and late-pregnancy revealed a higher inverse correlation between parathyroid hormone and vitamin D levels ( $r = -0.24$  and  $-0.27$ , respectively) (Hamilton et al., 2010; Brembeck et al., 2013), compared to what was found by Haddow et al. (2010). These correlations were also weaker than those found in the non-pregnant population (Aloia et al., 2006).

### **1.1.2 Vitamin D Deficiency During Pregnancy**

The best method for assessing vitamin D concentration in serum, is to measure the level of 25(OH)D, as this reflects the main circulating form of vitamin D, and is identified as the gold standard measurement (Van den Berg, 1997). This is also the best reflection of combined dietary vitamin D intake and vitamin D synthesis in the skin, and it is the substrate for both renal and non-renal synthesis of 1,25(OH)<sub>2</sub>D (Jones, 2008). The active form of vitamin D, however, is not used for measuring vitamin D status, due to its short half-life and lack of direct correlation with vitamin D status (Jones, 2008). In addition, the measurement of 1,25(OH)<sub>2</sub>D is more difficult than the measurement of 25(OH)D (Holick, 2003a).

#### ***1.1.2.1 Definition of Vitamin D Deficiency***

To prevent complications of 25(OH)D deficiency, it is recommended that people of all age groups have a serum level of more than 75 nmol/L (Heaney et al., 2003). Different cut-off points to describe hypovitaminosis D have been used in various population studies, but are not well-established and remain controversial. However, a 25(OH)D serum concentration of  $\geq 50$  nmol/L is said to be sufficient because it can maintain an optimal PTH concentration, and prevent increased bone calcium mobilisation (Malabanan et al., 1998). Hesitation over what constitutes sufficient serum vitamin D

concentration during pregnancy is even greater, and while the suggested values remain unvalidated in clinical trials, the debate will continue (Grant & Holick, 2005).

Vitamin D status in pregnancy has been classified into two categories based on sufficiency and deficiency;  $25(\text{OH})\text{D} \geq 50 \text{ nmol/L}$  ( $\geq 20 \text{ ng/mL}$ ) and  $25(\text{OH})\text{D} < 50 \text{ nmol/L}$  ( $< 20 \text{ ng/mL}$ ), respectively (Farrant et al., 2008; Cho et al., 2013; Parildar et al., 2013). Other similar cut-off points are used by the Endocrine Society (Holick, 2007; Holick et al., 2011b), and have been applied in previous pregnancy studies (Kramer et al., 2014; Lacroix et al., 2014; Arnold et al., 2015);

- Deficient levels:  $< 50 \text{ nmol/L}$  ( $< 20 \text{ ng/mL}$ )
- Insufficient levels:  $50\text{--}74.9 \text{ nmol/L}$  ( $20\text{--}29.9 \text{ ng/mL}$ )
- Sufficient levels:  $\geq 75 \text{ nmol/L}$  ( $\geq 30 \text{ ng/mL}$ ).

A slightly different classification, which has the additional category of severe deficiency (Hollis & Wagner, 2004; Dawodu & Wagner, 2007), has been applied to pregnant subjects in several studies (Flood-Nichols et al., 2015; Ross et al., 2011):

- Severely deficient levels:  $< 25 \text{ nmol/L}$  ( $< 10 \text{ ng/mL}$ )
- Deficient levels:  $25\text{--}49.9 \text{ nmol/L}$  ( $10\text{--}19.9 \text{ ng/mL}$ )
- Insufficient levels:  $50\text{--}74.9 \text{ nmol/L}$  ( $20\text{--}29.9 \text{ ng/mL}$ )
- Sufficient levels:  $75\text{--}200 \text{ nmol/L}$  ( $30\text{--}80 \text{ ng/mL}$ )

#### ***1.1.2.2 Prevalence of Vitamin D Deficiency***

Globally, the prevalence of vitamin D deficiency or insufficiency totals approximately one billion cases (Holick & Chen, 2008). Moreover, hypovitaminosis D is present in as many as 84% of pregnant women (Brannon & Picciano, 2011), depending on several factors, such as, country of origin and lifestyle (Holmes et al., 2009). According to the World Health Organization (WHO), the prevalence of vitamin D deficiency in pregnant women [ $25(\text{OH})\text{D} < 50$  and  $< 25 \text{ nmol/L}$ ] by region, was as follows: the Americas - 64%, 9%; Europe - 57%, 23%; Eastern Mediterranean - 79%, 46%; South-East Asia - 87%, not available, and Western Pacific - 83%, 13% (Saraf et al., 2015). Other studies

conducted in the Mediterranean region noted high a prevalence of hypovitaminosis D in gestation, reaching 90.3% (Karras et al., 2016).

Low vitamin D levels are not typically regarded as a potential problem in countries such as Saudi Arabia, due to the amount of sunshine it receives. However, some studies have suggested that up to 78% of Saudi's females, across all age groups, have low vitamin D levels (Al-Turki, 2008; Alfawaz et al., 2014). For example, one study conducted on 12,575 Saudi adolescents reported that 95.6% were vitamin D-deficient (AlBuhairan et al., 2015). Additionally, some studies have investigated women of childbearing age in Saudi Arabia, highlighting varying prevalence rates of vitamin D deficiency, ranging from one-third (Al-Turki, 2008) to two-thirds of the population studied (Kanan et al., 2013). Another recent study observed that 36.8 % of Saudi women aged 36 years exhibited vitamin D levels < 25 nmol/l (Al-Daghri et al., 2015).

To date, however, only limited studies have examined hypovitaminosis D in pregnant Saudi women (Serenius et al., 1984; Taha et al., 1984; Azhar, 2009; Aly et al., 2013; Al-Ajlan et al., 2015; Al-Faris, 2016; Al-Shaikh et al., 2016). Furthermore, the majority of these measured vitamin D just once, namely during the third trimester, or at the time of delivery, which does not reflect vitamin D levels during the entire pregnancy. However, Al-Faris (2016) did measure vitamin D in 160 pregnant women during the early stages of gestation, and noted that 50% had < 50 nmol/L, and 43.8% had 50-74 nmol/l.

**Table 1.2** presents the worldwide prevalence of vitamin D deficiency during gestation in different trimesters and countries. **Figure 1.2** shows the incidence of vitamin D deficiency around the world, both in pregnant women and in the general population (Hosseini-Nezhad & Holick, 2013).



**Table 1.2 Worldwide prevalence of Vitamin D deficiency during pregnancy**

Country	Year of publication	Trimester (gestational week)	Prevalence (n/N)	Reference
USA	2010	First trimester	33% (102/309)	Ginde et al. (2010)
	2015	First trimester <sup>a</sup>	10% (23.5/235) <sup>d</sup>	Flood-Nichols et al. (2015)
Canada	2011	First trimester (12-18)	39% (272/697)	Wei et al. (2012)
UK	2011	First trimester	57% (90/158)	Makgoba et al. (2011)
Spain	2016	First trimester	18% (366/2036)	Rodriguez et al. (2016)
UAE	2010	First trimester	78% (56/75)	Narchi et al. (2010)
Oman	2011	First trimester	98% (101/103)	Al Kalbani et al. (2011)
India	2017	First trimester	78% (214/275)	Kumari et al. (2017)
Finland	2011	First trimester	70% (481/686)	Miettinen et al. (2012)
Korea	2015	First trimester	91.8% (45/49)	Choi et al. (2015)
KSA	2015	First trimester <sup>a</sup>	68% (350/515)	Al-Ajlan et al. (2015)
	2016	First trimester	50% (80/160)	Al-Faris (2016)
Qatar	2013	Second trimester (> 24)	48% (907/1873)	Bener et al. (2013)
Spain	2011	Second trimester	59% (157/266)	Perez-Ferre et al. (2012)
USA	2013	Second trimester ( $\leq 26$ ) <sup>b</sup>	34.8% (713/2048)	Gernand et al. (2013)
China	2015	Second trimester	78.7 % (4583/5823)	Xiao et al. (2015)
	2017	Second trimester	77.4% (9288)	Zhao et al. (2017)
India	2009	Second trimester	74% (103/139)	Sahu et al. (2009)
Korea	2015	Second trimester	80.7% (67/83)	Choi et al. (2015)
Japan	2008	Third trimester (> 30)	90% (83/93)	Shibata et al. (2011)
Korea	2015	Third trimester	65.9% (58/88)	Choi et al. (2015)
China	2017	Third trimester <sup>c</sup>	78.8% (1393 /1768)	Zhao et al. (2017)
KSA	2013	Third trimester <sup>c</sup>	14.2% (13/92)	Aly et al. (2013)
	2016	At delivery	86.4 % (864/1000)	Al-Shaikh et al. (2016)
Egypt	2013	Third trimester ( $\geq 37$ )	40% (54/135)	El Rifai et al. (2013)
Turkey	2012	Third trimester ( $\geq 37$ )	90% (233/258)	Halicioglu et al. (2012)
Denmark	2010	Third trimester (39)	23% (32/141)	Milman et al. (2012)
New Zealand	2014	Third trimester (> 27)	57.7% (/150/260)	Grant et al. (2014)
Belgium	2012	First & third trimester	44.6% (585/1311)	Vandevijvere et al. (2012)
London	2013	At delivery <sup>a</sup>	36% (125/346)	McAree et al. (2013)
Kuwait	2005	At delivery	83% (178/214)	Molla et al. (2005)
Iran	2012	At delivery	74% (74/100)	Khalesi et al. (2012)
	2016	At delivery	48.9% (139/284)	Abbasian et al. (2016)
Tunisia	2016	At delivery	97% (84/87)	Ayadi et al. (2016)

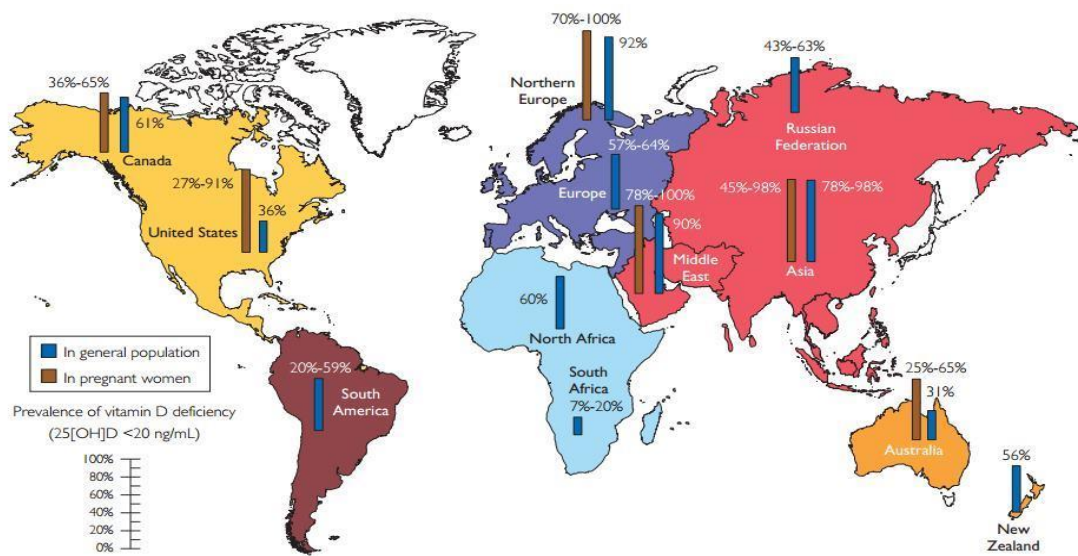
**Note:** Definition of Vitamin D deficiency - 25(OH) D < 50 nmol/L. KSA and UAE indicate Kingdom of Saudi Arabia and United Arab of Emirates, respectively.

<sup>a</sup> Vitamin D deficiency < 25 nmol/L

<sup>b</sup> Vitamin D deficiency < 37.5 nmol/L

<sup>c</sup> Vitamin D deficiency < 30 nmol/L

<sup>d</sup> the sample consisted of young nulliparous women.



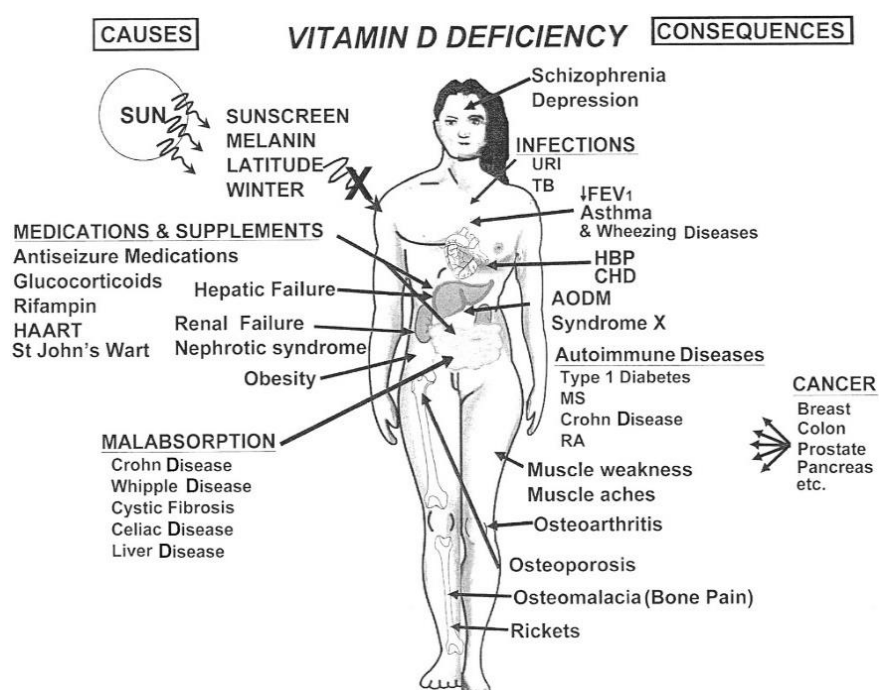
**Figure 1.2** Incidence of vitamin D deficiency around the world in pregnant women, and the general population (adapted from Hossein-Nezhad & Holick, 2013).

### 1.1.2.3 Adverse Health Effects of Vitamin D Deficiency

Hypovitaminosis D during gestation has been connected with multiple long-term health complications in both the mother and offspring (Barrett & McElduff, 2010). 25(OH)D levels during pregnancy may also predict levels in the neonate, as circulating vitamin D in the new-born is strongly correlated with levels in the mother, reaching up to 75% (Kovacs, 2013). Thus, early exposure to reduced vitamin D levels by the foetus may influence metabolic health and development.

It is known that a deprivation of 25(OH)D can lead to long-term complications, such as bone reabsorption, myopathy, inadequate calcium homeostasis (Mulligan et al., 2010), osteomalacia, and osteoporosis in adults (Stumpf et al., 2007; Yu et al., 2007). Maternal deficiency of vitamin D has also been linked with metabolic disorders, such as insulin resistance, GDM (Parlea et al., 2012), and pre-eclampsia (Wei et al., 2012). Additionally, it has been suggested that low vitamin 25(OH)D status elevates the risk of caesarean section (Perez-Ferre et al., 2012), and also predisposes the subject to bacterial vaginosis (Bodnar et al., 2009), pre-term delivery (Perez-Ferre et al., 2012), and infections (Kaludjerovic & Vieth, 2010). Short-term comorbidities of the foetus

with low concentrations of vitamin D include, impaired foetal growth, low infant birth weight and height (Haliloglu et al., 2011), low serum calcium, congenital rickets, abnormal tooth enamel formation, and lower bone mineral content (Haliloglu et al., 2011). Long-term foetal complications include skeletal problems, such as lower bone mineral density (Javaid et al., 2006) and immunomodulatory effects (Clifton Bligh et al., 2008). Other complications seen in children of deficient mothers include inflammatory and immunity disorders, such as type I diabetes mellitus (T1DM) (Barrett & McElduff, 2010), respiratory infections (Morales et al., 2012b), eczema, small size for gestational age (Bodnar et al., 2010), delayed mental and psychomotor development in infants (Morales et al., 2012a), language difficulties in children (Whitehouse et al., 2012), and infantile autism (Grant & Soles, 2009). Therefore, any risk factors that could potentially contribute towards deficiencies should be minimised to prevent complications.



**Figure 1.3** A schematic representation of the major causes of vitamin D deficiency and the potential adverse health effects (adapted from Holick & Chen, 2008). AODM, adult onset diabetes mellitus; CHD, coronary heart disease; FEV<sub>1</sub>, forced expiratory volume in 1 s; HAART, highly active antiretroviral therapy; HBP, high blood pressure; MS, multiple sclerosis; RA, rheumatoid arthritis; TB, tuberculosis; URI, urinary tract infection.

#### ***1.1.2.4 Risk Factors of Vitamin D Deficiency***

Multiple factors may increase an individual's susceptibility to vitamin D deficiency in different populations. The main risk factors of vitamin D deficiency are restricted sun exposure and reduced dietary intake, along with ethnicity, medication, smoking, and chronic diseases (Holick & Chen, 2008; Vandevijvere et al., 2012; Toher et al., 2014). Moreover, 25(OH)D status cannot be universally applied to all populations of different ages across varied geographical locations. This is because 25(OH)D levels are affected by distinct behavioural parameters associated with each population group. Thus, pregnant women may be vulnerable to low 25(OH)D levels due to increased maternal and foetal demands, haemodilution, and increased body fat (Wortsman et al., 2000; McAree et al., 2013).

One non-modifiable recognised risk factor for hypovitaminosis D in the pregnant and non-pregnant population is ethnicity. Some studies have highlighted the fact that pregnant women from ethnic minorities, such as African-Americans or Hispanics, are often deficient in vitamin D (Reeves et al., 2014). Pregnant women from Sub-Saharan Africa, the Middle East, and North Africa also tend to have low vitamin D levels, compared to their counterparts in other regions, indicating regional and ethnic variations in levels of vitamin D (Toher et al., 2014).

Several factors can limit sun exposure, thereby reducing the dermal production of vitamin D; these include age, skin colour, season, latitude, whole body coverage with clothing, exposure time, and daily sunscreen use in the pregnant and non-pregnant population (MacLaughlin & Holick, 1985; Holick, 1995b; Vercruyssen et al., 2012; Karras et al., 2014). Dark people absorb more UVB in the melanin of their skin than do white people and, therefore, require more sun exposure to produce the same amount of vitamin D (Holick, 1995b). A study conducted at Northern latitudes, namely in Sweden (latitudes 57-58°N) has proposed that during the winter, the majority of fair-skinned pregnant women suffer from vitamin D deficiency in their third trimester (Brembeck et al., 2013). When sun exposure is minimal, the dietary intake of vitamin D is essential to protect against such deficiency, with, some studies observing that low dietary intake

of vitamin D during pregnancy is associated with vitamin D deficiency (Vercruyssen et al., 2012; Karras et al., 2014). However, certain factors may alter vitamin D intake, such as the ingestion of substances that affect its absorption or metabolism, or the presence of certain kinds of disease, such as malabsorption, or liver and kidney disease (Holick, 2007). All of these may be considered classical risk factors for both the pregnant and non-pregnant population.

Some of the non-classical risk factors proposed by other studies, with regard to aggravating vitamin D deficiency, include disturbed inflammatory markers, obesity, physical inactivity, altered lipid profile, and multiparity (Wortsman et al., 2000; Tao et al., 2012; Cannell et al., 2014; Kluczynski et al., 2011; Andersen et al., 2013; Karras et al., 2014). Conversely, vitamin D plays an important role in the development of inflammatory diseases through its function in the immune system, whereby it inhibits the proliferation of pro-inflammatory cells and regulates the production of inflammatory cytokines (Haussler et al., 2008). However, the presence of inflammation may, in turn, indicate vitamin D deficiency. Prior studies have shown that the processes involved in contributing to chronic inflammatory disease appear to impact vitamin D levels (Autier et al., 2014). It has been suggested that as inflammation causes oxidative stress, this then disturbs the liver enzymes that are responsible for the synthesis of 25(OH)D, thereby reducing the levels of 25(OH)D (Mangge et al., 2015). Another possible explanation is that inflammation could induce metabolic clearance through the conversion of 25(OH)D to 1,25(OH)<sub>2</sub>D<sub>3</sub> as a means of controlling inflammation and self-healing, thus lowering the concentration of 25(OH)D (Cannell et al., 2014).

Numerous studies have been conducted on non-pregnant individuals to explore the correlation between vitamin D and inflammatory markers. For example, studies by Eleftheriadis and co-authors (2012) observed an inverse correlation between levels of vitamin D, c-reactive protein (CRP), and interleukin-6 (IL-6) in haemodialysis patients. Comparable findings have also been derived for individuals with diabetes undergoing coronary angiography (O'Hartaigh et al., 2013), and in asymptomatic adults (Amer & Qayyum, 2012).

A study on healthy subjects observed an occurrence of low vitamin D that was linked with higher leptin concentrations, however, no substantial correlations were seen between serum vitamin D and plasma concentrations of resistin and adiponectin (Vilarrasa et al., 2010). Studies on 25(OH)D concentrations and inflammatory markers during pregnancy, are in fact scarce, with some being undertaken on healthy pregnant women and others on pregnant women in poor states of health (Bodnar et al., 2007b; McManus et al., 2014; Bobbitt et al., 2015). One study performed on healthy African-American women determined a negative correlation between early-pregnancy vitamin D levels and pro-inflammatory cytokines, including TNF- $\alpha$ , Interleukin 1 beta (IL- $\beta$ ) and IL-6 in mid-pregnancy, after adjusting for confounding factors, such as the season (Bobbitt et al., 2015).

In addition, some studies conducted in pregnancy have shown that obese women are more likely to suffer from vitamin D deficiency than their non-obese counterparts (McAree et al., 2013; Karlsson et al., 2015), but others have failed to find an association in this regard (Choi et al., 2015; Flood-Nichols et al., 2015; Al-Faris, 2016; Ates et al., 2016). The studies claim that adiposity is a risk factor for vitamin D deficiency, and this is explained by the storage of 25(OH)D in adipose tissue through decreasing vitamin D bioavailability (Wortsman et al., 2000). Moreover, one study that measured fat mass percentage throughout all trimesters of pregnancy, deduced a significant inverse association with 25(OH)D exclusively in the first trimester, but failed to find this in the second and third trimesters (Karlsson et al., 2015). Furthermore, several studies conducted during pregnancy have revealed an inverse correlation between vitamin D and total cholesterol, low density lipoprotein cholesterol (LDL-cholesterol) (Tao et al., 2012), and a positive correlation with HDL-cholesterol (Makgoba et al., 2011).

Studies conducted on non-pregnant populations have explored physical activity and its relation to vitamin D status, revealing an association between outdoor physical activity and increased vitamin D levels (Scragg & Camargo, 2008; Brock et al., 2010; Kluczynski et al., 2011). More recently, several studies have observed that physical activity is related to higher vitamin D levels, independent of sun exposure in adults

(Touvier et al., 2015; Wanner et al., 2015), children, and adolescents (Al-Othman et al., 2012). However, Wanner et al. (2015) identified no significant association between vitamin D levels and outdoor physical activity, while a significant association was observed with indoor physical activity, indicating that other factors, independent of sun exposure, may play a role. One study followed up pregnant women from the first to third trimester, correcting for the season in terms of vitamin D, and noted that higher levels of physical activity were associated with increased 25(OH)D concentrations in early and late pregnancy (Moon et al., 2015). Furthermore, a German cross-sectional study revealed that physical inactivity was an important risk factor for low vitamin D levels, after controlling for season and independent risk factors, such as vitamin D supplementation, pre-pregnancy body mass index (BMI), intact parathyroid hormone, parity, and time spent outdoors (Wuertz et al., 2013).

Parity is another risk factor proposed in some studies. A positive relationship was found between multiparity and vitamin D deficiency, irrespective of the season (Andersen et al., 2013; Karras et al., 2014). All the above mentioned are risk factors for hypovitaminosis D during pregnancy, therefore, preventing these factors, while also accessing ample sources of vitamin D will help to maintain optimal 25(OH)D concentrations.

#### ***1.1.2.5 Vitamin D Requirements Met Through Sun Exposure and Food***

Sun exposure alone is sufficient to provide most people with their entire vitamin D requirements (Holick, 1995a). Sun exposure of 5-10 minutes, three times a week, between 11:00 am and 2:00 pm (noon), and at 42 degrees latitude will provide an individual with sufficient 25(OH)D, with minimal risk of skin damage (Holick, 2003a). To achieve this, the arms and legs, or face and arms should be sufficiently exposed (Holick, 2003a). When sun exposure is minimal, dietary intake of sufficient vitamin D appears to be crucial for avoiding vitamin D deficiency (Holick, 2011a). Additionally, the recommended daily allowance (RDA) of vitamin D during pregnancy, for maintenance of serum 25(OH)D concentration of 50 nmol/L, is 400-600 IU/day (IOM, 2010; Ross et al., 2011). Saudi Arabia has recently adopted vitamin D recommendations

from the USA, therefore, according to the Saudi Osteoporosis Society (SOS), supplementation of 600 IU/day is recommended for pregnant women (Al-Saleh et al., 2015).

In many countries, however, vitamin D intake from food is often lower than the recommended requirements. For example, in the US and Canada vitamin D intake in non-pregnant women is approximately 228 IU/day, and in the UK it is 88 IU/day. Meanwhile, in Europe it is 144 IU/day, and in Australia, 52 IU/day (Blumfield et al., 2012). In the USA during pregnancy, the average intake of vitamin D is estimated to be 225 IU from dietary sources and 319 IU from supplements (Camargo et al., 2007). However, daily intake for pregnant women in KSA has been reported to be less than 50% of the RDA (Ibrahim et al., 2013), which could contribute to low blood levels of vitamin D during pregnancy. Another study in early pregnancy reported that 91.9% of pregnant Saudi women ingested inadequate amounts of vitamin D, amounting to less than 600 IU/day (Al-Faris, 2016).

## **1.2 Obesity in Pregnancy**

### **1.2.1 Definition of Maternal Obesity**

Obesity is an international epidemic, and a major cause of illness and death worldwide (WHO, 2000). During pregnancy, a BMI of 30 kg/m<sup>2</sup> or more is considered obese (Modder & Fitzsimons, 2010). Women tend to have more body fat than men, so it is usually accepted that females with > 25 percent body fat are considered obese (WHO, 2000). However, there is no accepted definition for gestational obesity, due to the variability in weight gain, and the short duration of this status (Liat et al., 2015).

### **1.2.2 Components of Maternal Obesity**

During pregnancy, maternal weight is determined by multiple factors, such as socio-economic characteristics, lifestyle habits, metabolic rate, genetics, weight prior to pregnancy, and gestational weight gain (GWG) (Gaillard et al., 2013). This means that pregnant women are more vulnerable to obesity if they have a high BMI before



pregnancy, if they have gained more weight than is recommended during pregnancy, or both (Gore et al., 2003; Restall et al., 2014).

#### ***1.2.2.1 Gestational Weight Gain***

A normal pregnancy is accompanied by a normal weight gain increment (Rode et al., 2012). As pregnancy progresses, the amount of body fat increases, and approximately 30% of the GWG is attributed to increased adipose tissue (Devine et al., 2000). Although there are many studies addressing the determinants and outcomes of maternal weight gain, they are incapable of distinguishing the constituents of weight gain (fat mass vs. lean mass), or the effect of these constituents on the health of both the mother and the foetus. This could partially be explained by the difficulty of assessing the body composition of pregnant women, owing to the dynamic nature of gestation, and the absence of any methods for distinguishing between maternal and foetal components (Widen & Gallagher, 2014). The most frequently used method for assessing change to body composition in pregnant women, is through the anthropometrics of skinfold thickness and mid-upper arm circumference (MAC) (Widen & Gallagher, 2014).

GWG differs by trimester, but during the first trimester, it disproportionately constitutes fat (Devine et al., 2000). The second and third trimester are then characterised by fat accumulation, which influences subsequent insulin resistance in pregnant women, and therefore, progressive insulin resistance (Buchanan & Xiang, 2005; Hedderson et al., 2010). The pattern of maternal weight gain is greater in the second half of pregnancy, when, on average, it increases by 0.563 kg per week, with foetal weight strongly correlating with GWG during this period (Rasmussen et al., 2009).

In order to identify what is normal for GWG during pregnancy, in 1990 the IOM established recommendations for maternal weight gain in order to reaffirm ample weight gain. The aim was to avoid small-sized foetuses in relation to gestational age, as well as premature infants (IOM, 1990). Later in 2009, due to increased cases of being overweight or obese prior to pregnancy, the IOM recommendations for GWG were updated, with a modification concerning women's health outcomes in pregnancy. The

recommendations also aimed to reduce postpartum weight retention (PPWR) and obesity in childhood (Rasmussen & Yaktine, 2009). The WHO was the body responsible for reviewing recommendations for pregnancy weight in different trimesters, setting out range guidelines based on BMI prior to pregnancy (Rasmussen & Yaktine, 2009) (**Table 1.3**).

**Table 1.3 IOM recommendations for total, and rate of, weight gain during pregnancy, by pre-pregnancy BMI**

<b>Pre-pregnancy BMI</b>	<b>Total weight gain (kg)</b>	<b>Rates of weight gain in second and third trimester Mean (range) in kg/week</b>
Underweight women (< 18.5 kg/m <sup>2</sup> )	12.5-18 kg	0.51(0.44-0.58)
Women of normal weight (18.5-24.9 kg/m <sup>2</sup> )	11.5-16 kg	0.42 (0.35-0.50)
Overweight women (25.0-29.9 kg/m <sup>2</sup> )	7-11.5 kg	0.28 (0.23-0.33)
Obese women (≥ 30.0 kg/m <sup>2</sup> )	5-9 kg	0.22 (0.17-0.27)

**Note:** Calculations assume a 0.5-2 kg weight gain in the first trimester. BMI = kg/m<sup>2</sup>; independent of age, parity, smoking history, race, and ethnic background.

A recent study on Canadian women noted that 59.4% gained more than the recommended weight for their BMI during gestation (Dzakpasu et al., 2015), and excessive GWG has been reported in several industrialised countries, reaching as high as 74% (Rasmussen & Yaktine, 2009; Schiessl et al., 2009; Restall et al., 2014). However, to date, only a few studies have addressed GWG in Saudi Arabia (El-Gilany et al., 2011; Janbi et al., 2013). El-Gilany et al. (2011) observed that GWG was both below and above the IOM recommendations, at 16.4% and 47.3% of all pregnant women, respectively.

One study followed up a sample of women at 3 and 15 years postpartum, and revealed that women with adequate GWG gained an extra 3.06 kg compared to those with excessive GWG, who gained an extra 4.72 kg (Nehring et al., 2011). Therefore, excessive weight gain during pregnancy, may lead an individual to gain more postpartum weight and thus become overweight, and even obese (Mamun et al., 2010; Nehring et al., 2011). Hence, gestation is usually accompanied by additional weight

gain, as women tend to retain part of their weight gain in each gestation (Rode et al., 2012). Furthermore, excessive GWG from previous pregnancies can worsen gestational obesity (Cohen et al., 2014).

#### ***1.2.2.2 Pre-Pregnancy Weight***

A fundamental component of gestational obesity is pre-pregnancy BMI. Pre-pregnancy BMI in relation to gestational obesity is equivocal. One meta-analysis that studied pre-pregnancy BMI and PPWR, highlighted that the mean PPWR decreased in the group with increasing BMI (Rong et al., 2015). Elsewhere, it has been found that high pre-pregnancy BMI increases the possibility of maternal and infant health comorbidities (Doherty et al., 2006; Yan, 2015). Furthermore, the relationship between pre-pregnancy BMI and its effect on GWG is inconsistent; some studies found that mothers with excessive maternal weight gain were more likely to be overweight or obese prior to gestation, compared to expectant mothers of normal weight (Weisman et al., 2010; Restall et al., 2014). Other studies have highlighted that pregnant women with higher pre-pregnancy BMI experienced lower GWG (Nohr et al., 2008; Ebrahimi et al., 2015).

### **1.2.3 Prevalence of Maternal Obesity**

Obesity in pregnancy is a global burden, with variable prevalence (Heslehurst et al., 2010). Based on WHO statistics, Ono et al. (2005) state that “the rank order in Arabian countries for obesity in females is Kuwait (55.2%), Egypt (48%), and UAE (42%), which is higher than all the European countries and about the same as USA (48.3%) and Mexico (41%)”. They add that “countries such as Bahrain (37.9%), Jordan (37.9%), Saudi Arabia (36.4%) and Lebanon (27.4%) have higher obesity rates in females than UK (26.3%), Greece (26.4%), and Israel (25.9%)” (Ono et al., 2005). In KSA, the prevalence of obesity and being overweight is high in both Saudi males and females, reaching 40% (Al-Nuaim et al., 1997; Al-Nozha et al., 2005; Al-Baghli et al., 2008; Garawi et al., 2015). According to the WHO, 43% of the adult Saudi female population aged  $\geq 25$  years is obese (WHO, 2014), and among Saudi females of childbearing age, 31.5% are overweight, and 21.1% are obese (Al-Malki et al., 2003).

There are limited statistics documenting the indices for obesity in pregnant women worldwide, especially in the Middle East, but the prevalence varies globally between 1.8% and 25.3% (Guelinckx et al., 2008; Meher un et al., 2009; El-Gilany & Hammad, 2010; Bener et al., 2013). In the US and Australia, more than half of all prospective mothers are overweight or obese (Dodd et al., 2011; Flegal et al., 2012). In the UK, the prevalence of obesity in gestation reaches 19% (Kanagalingam et al., 2005; Shah et al., 2006; CMACE, 2010; Heslehurst et al., 2010). In Spain, specifically in the Canary Islands, 25% of pregnant women are recorded as being overweight, with 17.1% of these being obese (Bautista-Castaño et al., 2011). The proportion of overweight and obese pregnant women is 33% and 30% in Saudi Arabia, and 28.8% and 25.6% in Qatar (Nisa et al., 2009; Bener et al., 2013). A more recent study conducted on 14,568 pregnant Saudi women observed that the combined rates of pre-pregnancy overweight or obesity amounted to 68% of the sample population, and at  $\geq 28$  weeks of gestation, this increased to 69.7% (Wahabi et al., 2016).

#### **1.2.4 Adverse Health Effects of Maternal Obesity**

Obese pregnant women are identified as being at high risk because this health status may have major consequences for the mother, foetus, and child later in life (Weiss et al., 2004; Vesco et al., 2009; Nohr et al., 2012). The common complications noted in maternal obesity are metabolic, such as Hypertension (HTN), pre-eclampsia, GDM and PPWR (Weiss et al., 2004; Vesco et al., 2009). Obese pregnant women experience foetal overgrowth and a higher rate of delivery by caesarean section (Ehrenberg et al., 2004). Additionally, maternal obesity is accompanied by disturbances of inflammatory markers known as adipokines, and vascular dysfunction (Huda et al., 2010). Complications for the foetus include, increased prematurity, stillbirth, congenital malformation, and increased risk of infant mortality (Boots & Stephenson, 2011; Nohr et al., 2012; Gardosi et al., 2013). These problems may then lead to long-term complications, such as infant and childhood obesity, and T2DM later in life (Cedergren, 2006; Raatikainen et al., 2006).

### **1.2.5 Risk Factors for Maternal Obesity**

As mentioned earlier, the impact of pre-pregnancy weight on the risk of excessive GWG varies in the literature (Restall et al., 2014; Ebrahimi et al., 2015). Nevertheless, there are well-known risk factors of obesity, which are similar to the factors potentially provoking obesity during pregnancy, such as specific eating habits, a diet rich in fats and carbohydrates, and a sedentary lifestyle (Scholl et al., 1991; Olafsdottir et al., 2006; Ebrahimi et al., 2015). A limited number of studies have examined the relationship between food intake and GWG (Scholl et al., 1991; Olafsdottir et al., 2006; Ebrahimi et al., 2015). For example, Olafsdottir et al. (2006) claim that dietary energy intake is completely associated with excessive GWG, and this consists entirely of the total amount of protein, fat, and carbohydrate consumed, which leads to excessive GWG. Whereas, physical activity during pregnancy may reduce, or even prevent, this excessive weight gain (Streuling et al., 2011). Other classical risk factors of gestational obesity are age, a low educational level, multiparity, ethnicity, and genetic predisposition (Melzer & Schutz, 2010; Gaillard et al., 2013).

Some non-classical risk factors that may contribute towards obesity in pregnancy include, chronic inflammation and low vitamin D status. However, these are still debatable, and only a small number of studies have been conducted in this regard. Normal pregnancy is associated with both systemic and localised inflammation, either may enhance insulin resistance and cause hyperinsulinaemia, which will contribute to increased lipogenesis, and therefore, a susceptibility towards obesity (D'Anna et al., 2006; Sethi & Vidal-Puig, 2007; Yu et al., 2007; Karpe et al., 2011). This relationship will be discussed further, later in this thesis.

There is inconsistency in the research addressing vitamin D concentration and gestational obesity (Bodnar et al., 2007a; Josefson et al., 2013; McAree et al., 2013; Karras et al., 2016). A recent systematic review revealed a null association between 25(OH)D concentration and GWG, based on two studies conducted in the third trimester (Karras et al., 2016). However, a longitudinal study from the first to the third trimester identified that decreased levels of season-corrected 25(OH)D correlated with

higher GWG (Moon et al., 2015). This may occur as a result of low vitamin D status, resulting in elevated PTH, which in turn decreases 25(OH)D, and increases intracellular calcium in adipocytes. In this way, lipolysis is inhibited and lipogenesis is stimulated, resulting in obesity (Xue et al., 2001; McCarty & Thomas, 2003).

## **1.3 Gestational Diabetes Mellitus**

### **1.3.1 Background**

#### ***1.3.1.1 Definition of GDM***

According to the American Diabetes Association (ADA), GDM is defined as “carbohydrate intolerance resulting in hyperglycaemia of variable severity, with onset or first recognition during pregnancy” (Metzger et al., 1998). GDM is one of the most common comorbidities of pregnancy, usually diagnosed between 24 and 28 weeks of pregnancy (ADA, 2003). Although the mechanisms involved in its pathogenesis are still ambiguous, it is believed to be due to either insulin resistance, or impaired insulin secretion (Aguiree et al., 2013).

#### ***1.3.1.2 Pathophysiology of GDM***

The mechanisms involved in the aetiology of GDM appear to be multi-factorial (Buchanan et al., 2007; Arora & Hobel, 2010). The dysregulation of insulin secretion and resistance are important factors because they are altered by gestational and placental steroid hormones, and cytokine production (Fowler, 2007). Placental growth hormones, such as human placental lactogen, along with increased levels of cortisol and prolactin have been implicated in insulin insensitivity (Metzger et al., 2007; Reece et al., 2009). A lack of insulin resistance following delivery suggests a significant contribution of placental factors (Buchanan et al., 2007).

The low-grade inflammatory state that is associated with human pregnancy, can be attributed to cytokine production from different sources, including the placenta, uterine epithelium, uterine smooth muscles, cervix, immune system (T-lymphocytes and maternal and foetal macrophages), and adipose tissue, which increases the complexity

of the immune-metabolic network in pregnant women (Arora, 2011). Both adipose tissue and the placenta can produce inflammatory markers, such as leptin, adiponectin, resistin, TNF- $\alpha$ , and IL-6, which are collectively known as adipokines, or adipocytokines (Desoye & Hauguel-de Mouzon, 2007; Miehle et al., 2012). Thus, normal pregnancy is associated with a state of systemic inflammation that can activate defined inflammatory pathways, which could be vital for inducing the insulin resistance necessary for the progression of a normal pregnancy (Radaelli et al., 2003; Koerner et al., 2005; Desoye & Hauguel-de Mouzon, 2007; Gomes et al., 2013). The indicators of systemic inflammation, associated with pregnancy, are higher levels of serum CRP and/or IL-6 (D'Anna et al., 2006; Yu et al., 2007). As described earlier, the combination of systemic and localised inflammation may result in pregnancy complications, such as diabetes, obesity, and pre-eclampsia (Lepercq et al., 1998; Benyo et al., 2001; Coughlan et al., 2001; Romero et al., 2007).

Conversely, there is growing evidence to suggest that the dysregulation of beta cells ( $\beta$ -cells), in response to increased insulin resistance, leads to GDM (Buchanan et al., 2007; Fowler, 2007). Moreover, the increased need for insulin during gestation places an extra burden on the pancreatic  $\beta$ -cells, contributing to further dysregulation of insulin secretion (Buchanan et al., 2007). Inflammatory proteins are also implicated in  $\beta$ -cell damage and insulin resistance (Vrachnis et al., 2012), while genetic mutations and autoimmune destruction may be responsible for  $\beta$ -cell dysfunction, thus contributing to the induction of GDM (Buchanan et al., 2007). Moreover, insulin resistance may be ascribed a dysfunction of the insulin receptors (Desoye et al., 1994; Desoye et al., 1997).

As already mentioned, a vitamin D deficiency has been linked to the induction of GDM (Alvarez & Ashraf, 2009; Eliades & Pittas, 2009). Vitamin D is considered an anti-inflammatory vitamin (Bikle, 2009), and low levels have also been implicated in insulin receptor dysfunction, thus contributing towards the cause of GDM (Desoye et al., 1994, 1997). Together, hypovitaminosis D and insulin receptor dysfunction contribute to the inflammatory effect, which is exacerbated by maternal factors, such as obesity and a genetic predisposition towards GDM (Kahn et al., 2006; Arora, 2011). As a result, there

is a growing body of evidence indicating that vitamin D deficiency can impact upon metabolic diseases, such as GDM. Although, further data on the role of vitamin D in GDM is required (Zhang et al., 2008; Arnold et al., 2015).

### ***1.3.1.3 Screening and Diagnostic Criteria for GDM***

The screening and diagnosis of GDM are of paramount importance for preventing complications associated with GDM (Taylor & Burley, 2015). However, there is no consensus on a unified approach to diagnosing GDM by screening patients, whether based on the selective screening for risk factors, or the universal screening of all pregnant women (O'Dea et al., 2014). Similarly, the adoption of globally accepted diagnostic guidelines for GDM screening has also been a subject of debate (Agarwal et al., 2005). In order to attain a single approach for GDM diagnosis worldwide, in 2010 the International Association of Diabetes and Pregnancy Study Group (IADPSG) issued consensus guidelines for the consideration of glycaemia and pregnancy outcomes (Panel IADPSG Consensus, 2010). The corresponding data were obtained from the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study, which was conducted in 15 field centres, across nine different countries, along with other important observational epidemiological and randomised controlled trials (RCT).

The IADPSG diagnosis criteria stipulates that GDM rates will increase, as evidenced by the HAPO study, which saw the prevalence rate of GDM increased from 11.3% to 16.1%, a 42% increment (Metzger et al., 2008). Thus, the recent IADPSG and WHO 2013 diagnostic criteria recommend the inclusion of all high-risk pregnant women, resulting in higher percentages of women with GDM being reported (Panel IADPSG Consensus, 2010). Hence, women are now more aware of their high-risk status during pregnancy, which will help prevent pregnancy complications.

Numerous studies in different countries have revealed an increase in prevalence upon the adoption of the IADPSG criteria, as shown in **Table 1.4**. The New Zealand Society for the Study of Diabetes (NZSSD) reported a 6% prevalence of GDM, whereas using the IADPSG criteria, this increased to an estimated prevalence of 9.7% (Ekeroma et al.,



2015). Similarly, in the UAE, and using ADA criteria, of GDM was found to be 12.9%, as opposed to 37.7% reported using the IADPSG criteria (Agarwal et al., 2010). From previous studies, along with Jenum et al. (2012), it may be observed that the prevalence of GDM in a multi-ethnic population is 2.4 times greater using the altered IADPSG criteria, compared with the WHO criteria (Jenum et al., 2012). On a wider scale, national and international bodies, whether wholly or in substantial part, have endorsed the IADPSG, including the ADA, the Endocrine Society, and the WHO. This has led to the issue of guidelines for the diagnosis of hyperglycaemia in pregnancy, with a contribution made to the standard diagnostic approach to GDM (Blumer et al., 2013; WHO, 2013). However, certain other bodies, such as the American College of Obstetricians and Gynaecologists (ACOG) still favour other diagnostic criteria (ACOG, 2013) along with the ADA which proposed in 2014 that either the IADPSG or ACOG could be acceptable (ADA, 2014).

### **1.3.2 Prevalence of GDM**

The global prevalence of GDM in the population ranges between 1% and 14% of all pregnancies (Krishnaveni et al., 2007; Jang, 2011). The IADPSG has reported the overall prevalence of GDM to be between 9.3% and 25.5%, thus averaging 17.8% (Sacks et al., 2012). In the US, the prevalence of GDM ranges from 3.47 to 7.15% depending on the State, and in Canada, the prevalence is 17.8% (Ryan, 2011; Bardenheier et al., 2013). In contrast, the GDM prevalence in Middle Eastern countries ranges from 13.9% in Qatar to 7% in Iran (Maghbooli et al., 2008; Bener et al., 2013). However, in 2015, one study in Saudi Arabia reported a 39.4% prevalence of GDM, among all pregnancies in the Kingdom (Alfadhli et al., 2015).

**Table 1.4** shows GDM prevalence across these and other countries. However, the variation observed may be due to differences in diagnostic approaches and ethnic diversity (Krishnaveni et al., 2007).

**Table 1.4 Worldwide prevalence of GDM, especially in the Middle East**

Country	Year	Prevalence (n/N)	Criteria	Reference
USA	2013	3.47 to 7.15% (620332 to 1278207/1,787,703)	Different criteria in different States	Bardenheier et al. (2013)
Canada	2011	17.8% (4,150/ 23,316)	IADPSG	Ryan (2011)
Ireland	2011	12.4% (682/5,500) 9.4% (517/5,500)	IADPSG WHO 1999	O'Sullivan et al. (2011)
New Zealand	2015	9.7% (619/6376) 6% (381/6376)	IADPSG NZSSD	Ekeroma et al. (2015)
France	2013	14.4% (2703/18,775)	French and/or WHO recommendations	Cosson et al. (2013)
Germany	2011	2.3% (14,990/647,385)	Not clear	Schneider et al. (2011)
	2016	6.81% (10,816 /158,839)	IADPSG	Tamayo et al. (2016)
Australia	2011	9.6% (122/1275) 13.0% (166/1275)	ADIPS criteria IADPSG	Moses et al. (2011)
India	2013	11% (55/500)	WHO 1999	Sharma et al. (2013)
	2017	17% (71/417)	IADPSG	Sharma et al. (2017)
North India	2015	9% (459/5100) 35% (1785/5100)	WHO 1999 WHO 2013	Arora et al. (2015)
Asia	2014	28.8% (246/855) 21.1% (180/855)	WHO 1999 WHO 2013	Yew et al. (2014)
	2011	6.8% (7172/105 473)	WHO 1999	Zhang et al. (2011)
China	2011	8.9% (1293/14593) 14.7% (2138/14 593)	NDDG IADPSG	Wei & Yang (2011)
	2013	17.5% (3007/17,186)	IADPSG	Zhu et al. (2013)
	2015	8.1% (1505/18589) 9.3% (1728/18589)	WHO 1999 IADPSG	Leng et al. (2015)
Saudi Arabia	2010	12.5% (96/770) 3.8% (29.3/770)	WHO 1999 ADA	Al-Rowailly & Abolfotouh (2010)
	2013	18% (569/3157)	ADA	Wahabi et al. (2014)
	2015	39.4% (183/445)	IADPSG	Alfadhli et al. (2015)
	2015	13.8% (238/1718)	Different criteria	Serehi et al. (2015)
	2016	24.2% (2355/9731)	WHO 2013	Wahabi et al. (2016)
	2016	11.1% (111/1000)	WHO 1999	Al-Shaikh et al. (2016)
UAE	2010	12.9% (1,328/10,283) 37.7% (3,875/10,283)	ADA IADPSG	Agarwal et al. (2010)
Bahrain	2012	10.1% (5004/49 552)	National Diabetes Data Group, 1979	Rajab et al. (2012)
Qatar	2011	16.3% (335/2056)	WHO 1999	Bener et al. (2011)
	2013	13.9% (260/1873)	WHO 1999	Bener et al. (2013)
Oman	2002	21.3% (120/564)	ADA	Dashora et al. (2002)
Yemen	2016	5.1% (16/311)	ADA	Ali et al. (2016)
Nigeria	2015	3.8% (40/1059) 8.1% (86/1059) 8.6% (91/1059)	WHO 1999 WHO 2013 IADPSG	Olagbuji et al. (2015)
Turkey	2012	8.1% (65.5/808) 5.6% (45/808) 15.7% (127/808)	C&C NDDG New criteria	Kösüs et al. (2012)
	2015	4.3% (39/815)	C&C	Erem et al. (2015)
Iran	2015	4.9 % (62.5/1276)	C&C	Mohammadzadeh et al. (2015)
	2016	29.9% (224/750)	IADPSG	Shahbazian et al. (2016)

**Note:** Australasian Diabetes in Pregnancy Society (ADIPS) criteria; American Diabetes Association (ADA); National Diabetes Data Group (NDDG); Carpenter and Coustan (C&C), and the New Zealand Society for the Study of Diabetes (NZSSD).

### **1.3.3 Adverse Health Effects of GDM**

GDM has been implicated in both long- and short-term health complications in mothers and their offspring. The short-term effects of GDM among expectant mothers include maternal HTN, pre-eclampsia, urinary tract infections, and caesarean delivery (Getahun et al., 2010; Catalano et al., 2012). On the other hand, the long-term maternal complications include a 35-80% higher possibility of recurring GDM (Ben-Haroush et al., 2004) and 7-8 times higher risk of T2DM later in life (Bellamy et al., 2009). The offspring of mothers with GDM, also tend to have higher blood glucose levels, amino acids, and lipids, due to the transplacental transfer from the mother, along with elevated insulin (Catalano & Ehrenberg, 2006). This in turn leads in changes in foetal body composition and metabolism (Catalano & Ehrenberg, 2006). The short-term complications for offspring of mothers with GDM include macrosomia (Alberico et al., 2014), birth trauma, congenital malformation, respiratory distress syndrome (Chen et al., 2009), neonatal jaundice (Sobande et al., 2005), and perinatal morbidity and mortality (Bellamy et al., 2009). Whereas the long-term complications include, childhood obesity, impaired glucose tolerance, T2DM (Boney et al., 2005; Ferrara, 2007; Hiersch & Yogev, 2014), and cardiovascular diseases (Chen et al., 2009).

GDM accounts for an increase of 636 million United states dollar (USD) in national medical costs (Chen et al., 2009), and in 2013, the global health expenditure on the treatment of diabetes and the management of its health effects totalled 548 billion USD (Aguiree et al., 2013). Therefore, by gaining further understanding and minimising the high-risk factors of GDM, the prevalence can be reduced, and hence the cost of treating its complications.

### **1.3.4 Risk Factors of GDM**

The well-established risk factors of GDM include obesity, maternal age > 35 years, past GDM, and a family history of GDM and/or T2DM. Other risk factors are ethnicity, multiparity, history of foetal death, history of prior caesarean section, history of a macrosomic infant, low birth weight, recurrent miscarriages, presence of glycosuria, alcohol consumption, short stature, cigarette smoking, low physical activity before or

during pregnancy, excessive GWG, poor diet, socio-economic factors, HTN, genetic susceptibility to polymorphism, and diagnosis of polycystic ovarian syndrome (PCOS) (Dode & Santos, 2009; Al-Rowaily & Abolfotouh, 2010; Lewis et al., 2010; Zhang & Ning, 2011; Nankervis et al., 2012; Erem et al., 2015).

However, the known risk factors only contribute to approximately 50-60% of the risk of future GDM (Savvidou et al., 2010). Ethnicity is believed to be a strong and non-modifiable risk factor (Yuen & Wong, 2015), with GDM prevalence being especially high among women from Hispanic, Native American, African-American, Pacific island, indigenous Australian, and South or South East Asian backgrounds (Kjos & Buchanan, 1999).

Obesity during pregnancy is considered to be a well-known independent risk factor of GDM (Teh et al., 2011; Singh et al., 2012; Mao et al., 2015), and pre-pregnancy obesity has a strong direct correlation with GDM prevalence (Li et al., 2013; Gabbay-Benziv et al., 2015). Erem et al. (2015) demonstrated that women who were obese pre-pregnancy were up to 60 times more likely to develop GDM than underweight women (Erem et al., 2015). In the first trimester of pregnancy, obesity proxies, such as visceral fat mass percentage, have been shown to positively correlate with hyperglycaemia, insulin resistance, dyslipidaemia, and high diastolic blood pressure (Gur et al., 2014). Similarly, BMI, subcutaneous fat, and serum leptin have been shown to have a direct correlation with GDM (Sommer et al., 2015). In addition, waist to hip ratio (WHR) in early pregnancy has been found to be significantly correlated with GDM at 7-12 weeks of gestation (Alptekin et al., 2016).

Nevertheless, it must be taken into account that the results of studies addressing maternal weight gain and GDM risk have not always been consistent. For instance, some have addressed early GWG before GDM screening, and observed that it is an independent risk factor of GDM (Saldana et al., 2006; Hedderson et al., 2010; Gaillard et al., 2013; Sommer et al., 2014; Zheng et al., 2014; Erem et al., 2015; Mao et al., 2015). In 2015, two meta-analyses noted that excessive GWG (based on IOM) occurring prior to the diagnosis of GDM, increased the risk, even after adjusting for

confounding factors (Brunner et al., 2015; Robitaille, 2015). In contrast, other meta-analyses have failed to demonstrate an association between GWG and GDM (Ruifrok et al., 2014). Furthermore, another set of studies noted an inverse association, i.e. maternal weight gain was lower in GDM women compared to women without GDM (Catalano et al., 1993; Nohr et al., 2008; Heude et al., 2012). These differences may be due to cut-off point used, maternal body weight, the timing of the measurements, for example, the GWG measured was the total weight gain over the entire pregnancy. This could have been affected by either behavioural or treatment changes after diagnosis, thus influencing weight increase and foetal weight (Dode & dos Santos, 2009; Hedderston et al., 2010; Zheng et al., 2014).

Physical inactivity in non-pregnant individuals is a well-known major risk factor for the development of diabetes. The health benefits of physical activity are well documented, and include reducing weight gain and decreasing insulin resistance (Jeon et al., 2007). Physical inactivity prior to pregnancy, or in the first trimester of pregnancy, has been shown to be a significant risk factor for the development of GDM in late pregnancy (Harizopoulou et al., 2010). A prospective cohort study of Pakistani women who were  $\leq 18$  weeks pregnant, revealed that an increased risk of GDM was directly associated with physical inactivity (Iqbal et al., 2007). A number of observational studies have demonstrated that regular physical activity, both prior to and during the first trimester of pregnancy, will reduce the risk of GDM (Dempsey et al., 2004; Oken et al., 2006; Zhang et al., 2006; Liu et al., 2008; Mørkrid et al., 2014).

Other non-traditional risk factors that could play a role in GDM development, include higher levels of triglycerides (TG) (Brisson et al., 2010), low vitamin B12 (Sukumar et al., 2015), vitamin D deficiency (Zhang et al., 2008; Lau et al., 2011; Parlea et al., 2012) and high PTH in small studies (Kramer et al., 2014); all of which have been independently linked with higher GDM risk. Pregnancy is normally associated with insulin resistance, along with altered maternal lipid metabolism in the latter half of pregnancy (Alvarez et al., 1996). There are reports documenting that TG levels increase progressively during pregnancy, with significantly higher levels in the later stages of gestation (Alvarez et al., 1996; Herrera & Ortega-Senovilla, 2010). Elevated levels of

TG, total cholesterol and LDL-cholesterol, across the three trimesters, have also been reported among women with GDM, compared to their counterparts without GDM (Ryckman et al., 2015). However, other studies that have measured lipid levels in early pregnancy, with the risk of insulin resistance later in pregnancy and GDM occurrence, are still debatable and unclear (Nolan et al., 1995; Zhou et al., 2012; dos Santos-Weiss et al., 2013; Ryckman et al., 2015). Shaung & Huixia (2014) revealed that high serum TG levels in early pregnancy are an independent risk factor for GDM. Furthermore, one study noted that pregnant women with higher TG levels, irrespective of their BMI, were at greater risk of developing GDM, while lean women with high HDL-cholesterol were protected (Li et al., 2015).

In addition, low vitamin D levels have been shown to increase the risk of GDM in several meta-analyses (Aghajafari et al., 2013; Wei et al., 2013), which has been attributed to the presence of VDRs in the pancreatic cells. Therefore, vitamin D plays a role in insulin resistance, stimulating insulin secretion, and in turn glucose homoeostasis (Alzaim & Wood, 2013). Several observational studies have measured vitamin D levels, either after GDM occurrence in the second or third trimester (Zuhur et al., 2013; Burris & Camargo, 2014), or in early pregnancy before GDM diagnosis (Lacroix et al., 2014; Jain et al., 2015), and found that hypovitaminosis D increases the risk of GDM. RCT studies are limited, but fundamental to gaining an understanding of the role of vitamin D in decreasing the risk of GDM, or enhancing glucose tolerance in pregnant women with GDM (Burris & Camargo, 2014). Interestingly, Zuhur et al. (2013) highlighted, amongst women in their second trimester of pregnancy, that severe vitamin D deficiency is independently associated with GDM, along with raised PTH levels, which were significantly greater in women with GDM. Thus, vitamin D deficiency and elevated PTH levels may place an additional burden on glucose tolerance during pregnancy.

## **1.4 Study Hypothesis and Aims**

The current study hypothesis is that a vitamin D deficiency, early in pregnancy has a deleterious impact on the health of pregnant women, due to an increased risk of metabolic disease. To test this hypothesis, the present study measured serum vitamin D twice in a sample of pregnant Saudi women: testing once in early pregnancy, then a second time in mid-pregnancy to assess the onset of GDM, along with its markers. These measurements, in addition to a detailed characterisation of all other confounding factors, may affect the independent association between vitamin D deficiency and GDM, such as parity, education, income, season, pre-pregnancy BMI, GWG, dietary factors (food and supplements), physical activity, sun exposure, skin colour, age, family history of diabetes, past GDM, ethnicity, and health status. These extensive measurements provide a more holistic approach to understanding how pregnancy influences vitamin D. To explore this understanding; the following aims were addressed:

1. Determining the prevalence and determinants of vitamin D deficiency in early pregnancy, amongst women in Saudi Arabia.
2. Comparing the vitamin D levels and risk factors that change from early to mid-pregnancy.
3. Assessing the prevalence and determinants of GDM in Saudi population.
4. Exploring possible independent association of vitamin D deficiency in early pregnancy and GDM risk with adjustment for confounding factors.

## **Chapter 2**

### **Materials and Methods**

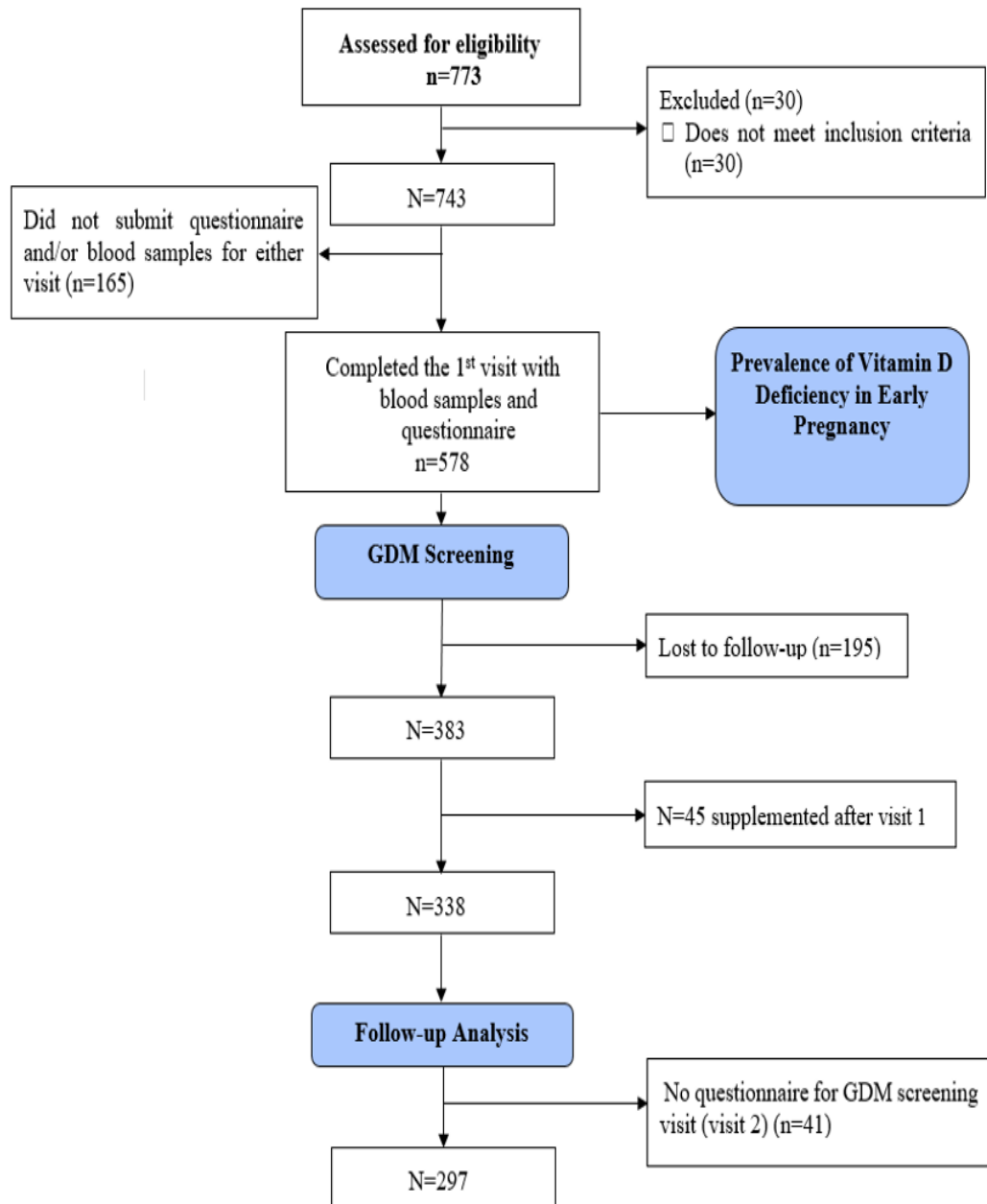


## 2.1 Research Methodology

### 2.1.1 Study Design

A total of 773 pregnant women, aged 18-39 years, were assessed for eligibility. Thirty were subsequently excluded from the study because they did not meet the inclusion criteria; 27 were taking vitamin D supplements, and 3 had diabetes mellitus type I or II. In total, 743 pregnant women assessed during the first trimester of pregnancy (8-12 weeks) were eligible to proceed in the study. Out of these, 578 completed the questionnaire for the 1<sup>st</sup> visit, with a blood sample. The mean age of these subjects was  $28.8 \pm 5.4$  years. At the 2<sup>nd</sup> visit (24-28 weeks), 281 were lost to follow-up [refusal to consent to a glucose tolerance test (n = 41); refusal to complete a questionnaire at the 2<sup>nd</sup> visit (n = 41); spontaneous abortion (n = 34); vitamin D supplementation after the 1<sup>st</sup> visit (n = 45); cancellation of appointments for a variety of reasons at the time of the GDM screening (n = 120)]. These follow-up losses included Corona cases (viral respiratory disease) at King Khalid University Hospital (KKUH), which caused the outpatient department to close for a month, and follow-ups elsewhere. Finally, a total of 297 pregnant women were fully assessed at the 2<sup>nd</sup> visit.

The present study was a prospective observational study of 297 pregnant women, with a mean age of  $28.9 \pm 5.3$  years. The data were collected from three tertiary antenatal clinics in Riyadh, Saudi Arabia (latitude: 24° 42' N, 46° 43' E). The study was accepted by the institutional review boards of King Fahd Medical City (KFMC) and KKUH, in Riyadh ( **Appendix I**). Each pregnant woman gave informed consent for their participation ( **Appendix II**). The Consolidated Standard of Reporting Trials (CONSORT) form (**Figure 2.1**) describes the flow of participants through the study.



**Figure 2.1** CONSORT statement describing flow of participants through the study.

## **2.2 Sample Population**

### **2.2.1 Inclusion and Exclusion Criteria**

Pregnant Saudi women, aged 18-39 years prior to 16 weeks of gestation, were involved in the current study. Singleton pregnancy was another criterion for inclusion, together with no previous history of diabetes mellitus (type I or II). Exclusion criteria included non-Saudi subjects, a gestational age of over 16 weeks, vitamin D supplementation one month prior to pregnancy, previous history of diabetes mellitus, calcium or parathyroid disorders, treatment with cardiac medication or diuretics, and women with chronic HTN or malabsorption syndrome. Pregnant women with chronic conditions, such as thyroid disease, epilepsy, or malignancy were also excluded.

### **2.2.2 Power of the Study**

At the time of this study, there were no prospective data on pregnant Saudi women with GDM, and the association of this with vitamin D, so no direct power calculation could be performed. Therefore, in order to show vitamin D deficiency as a risk factor for GDM in pregnant women, a relative risk of 3.0 at 5% level of significance and 80% power was calculated, similar to a previous prospective study (Zhang et al., 2008). This indicated a 30% incidence of GDM among vitamin D deficient pregnant women and 10% incidence of GDM without vitamin D deficiency. For this power, it was observed that 250 pregnant women would need to be recruited for the study. Assuming a 10% drop-out rate, the estimated sample size was 275. However, another interest of the study was the prevalence of vitamin D deficiency among pregnant Saudi women. Assuming a prevalence of 80% vitamin D deficiency (Azhar, 2009), at a 5% level of significance and with  $\pm 5\%$  level of precision [width of 95% CI], 246 pregnant women were required. The total sample size for the above study was ultimately 297, and this is a similar number of participants used in some of the studies conducted previously (Makgoba et al., 2011; Arnold et al., 2015).

## 2.3 Recruitment and Medical Screening

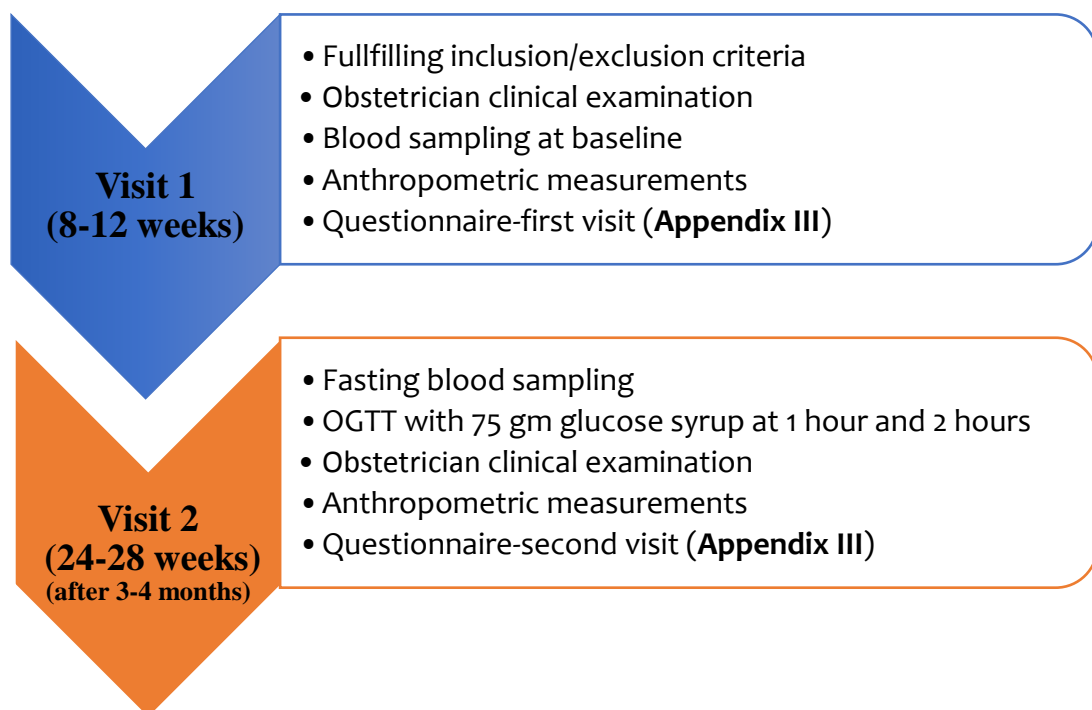
Banners and brochures were placed in prenatal clinics in all three of the hospitals sampled: KKUH, KFMC, and Prince Salman Hospital. Additionally, obstetricians were asked to introduce the research to their pregnant patients at their first prenatal appointment. Patients who met the criteria and consented to contribute in the present study were given the appropriate information. At their early pregnancy visit, prospective candidates were asked to sign consent forms that included information about their participation in the study, such as data collection, procurement of blood samples, and anthropometric measurements. Their permission for data collection from their medical records and blood stocks in a bio-bank (**Appendix II**) were also obtained. Moreover, the participants were informed of their freedom to withdraw from the study at any point, without it affecting their usual care. Finally, a questionnaire specifically designed for data collection was used to collect the relevant information, such as socio-economic information, medical history, diet, sun exposure, and levels of physical activity (**Appendix III**).

## 2.4 Study Visits

Clinical assessments were conducted twice during the early and mid-pregnancy visits. In early visit baseline, non-fasting blood samples were obtained (8-12 weeks); lipid profile and 25(OH)D levels were measured, gestational age was calculated from the last menstrual period (LMP), and the GDM screening in mid-pregnancy visit was scheduled during the second trimester (at 24-28 weeks). For the oral glucose tolerance test (OGTT), the women were asked to fast for at least 10 hours, and a fasting blood sample was subsequently withdrawn, following this, the participants were asked to drink 75 gm of glucose within a period of five minutes. Blood samples were collected after one, and then after two hours, for assessment of glucose and insulin levels.

IADPSG guidelines were applied to diagnose GDM, based on one or more values equal to, or exceeding, the following threshold: fasting  $\geq 5.1$  mmol/L and/or 1-hour post glucose  $\geq 10$  mmol/L and/or 2-hour post-glucose load  $\geq 8.5$ mmol/L (Panel IADPSG

Consensus, 2010). During both visits, maternal health status was assessed by the obstetrician for acute illnesses, such as viral and/or bacterial gastrointestinal and respiratory diseases. Anthropometric assessment and questionnaire-based interviews were also conducted. These visits were arranged alongside obstetric appointments for the sake of the participants' convenience, and each was assigned to a unique number and file, for data storage purposes.



**Figure 2.2** The follow-up visits

## 2.5 Data Collection

Data were collected between January 2014 and December 2015. The study tools included anthropometric measurements, blood biochemical tests, and an interview questionnaire. The interview questionnaire included questions to gather socio-economic information, clinical measurements, and past medical and treatment history. It also contained a food frequency questionnaire (FFQ) and questions on sun exposure and physical activity (Al-Daghri et al., 2011; Harrison et al., 2011; Al-Musharaf et al.,

2012; Al-Othman et al., 2012b; Takahasi et al., 2013). A pilot study was conducted, to test the clarity and efficiency of the questions, on 62 subjects. **Table 2.1** summarises the measurements and tools used.

**Table 2.1 Summary of the sampling and measurements in the study**

	<i>Method or Sample Used</i>	<b>8-12 weeks</b>	<b>24-28 weeks</b>
<b><i>Maternal Anthropometrics</i></b>			
Weight (kg)	Digital Pearson Scale (twice)	✓	✓
Pre-pregnancy weight (kg)	Subject recall	✓	
Height (cm)	Digital Pearson Scale (twice)	✓	
Waist (cm)	Non-stretchable tape (twice)	✓	✓
Hips (cm)	Non-stretchable tape (twice)	✓	✓
MAC (cm)	Non-stretchable tape (twice)		
Skinfold thickness (mm)	Caliper	✓	✓
Blood pressure (mmHg)	Mercury sphygmomanometer (twice)	✓	✓
<b><i>Socio-Economic Data</i></b>	Questionnaire	✓	
<b><i>Clinical Measurements</i></b>			
Family history	Questionnaire	✓	
Subject history	Questionnaire	✓	
Risk factors	Questionnaire	✓	
Pregnancy symptoms	Questionnaire	✓	✓
Pregnancy complications	Obstetric assessment	✓	✓
List of medication	Questionnaire	✓	
<b><i>Dietary Assessment</i></b>			
FFQ	Questionnaire	✓	✓
Supplement intake	Questionnaire	✓	✓
<b><i>Other</i></b>			
Sun exposure	Questionnaire	✓	✓
Physical activity	IPAQ	✓	✓
<b><i>Biochemical Assessment</i></b>			
- 25(OH) D, lipid profile, calcium, phosphorus, albumin, creatinine, alkaline phosphatase, blood glucose, insulin	Serum sample	✓	
- HbA1c	Whole blood	✓	✓
- 25(OH)D, lipid profile, calcium, phosphorus, albumin, creatinine, alkaline phosphatase, fasting blood glucose, fasting insulin	Fasting serum sample		✓
- 1- and 2-hour OGTT and 2-hour insulin	1- and 2-hour serum sample		✓
-HOMA-IR, HOMA-β	Matthews equations		✓

**Note:** Mid-upper arm circumference (MAC); International Physical Activity Questionnaire (IPAQ); Oral glucose tolerance test (OGTT); Homoeostasis Model Assessment of Insulin Resistance (HOMA-IR) and beta cell (HOMA-β).

### 2.5.1 Anthropometric Measurements

Anthropometric measurements were taken from the participants during early and mid-pregnancy. This included weight (kg) and height (cm), for calculating BMI ( $\text{kg/m}^2$ ); pre-pregnancy weight (kg); pre-pregnancy BMI ( $\text{kg/m}^2$ ); GWG (kg); waist (cm) and hip circumference (cm), for waist-hip ratio (WHR); mid-arm circumference (MAC); triceps, biceps, suprailiac and subscapular (mm) measurements (skinfold thickness), and relative fat percentage. In addition, systolic and diastolic blood pressure (mmHg) was also recorded.

Weight was documented to the nearest 0.1 kg, using an appropriate international standard scale (Digital Pearson Scale, ADAM Equipment Inc., USA), without shoes and with lightweight clothing. Height was measured at the early pregnancy visit only. This was recorded to the nearest 0.5 cm, using the Digital Pearson Scale, while standing upright without shoes. Measurements were taken twice, and the mean readings were recorded. Pre-pregnancy BMI was calculated from weight recall, which was categorised based on WHO (2000) guidelines. To minimise bias in recalling pre-pregnancy weight, the study showed a strong correlation between pre-pregnancy BMI and BMI at the 1<sup>st</sup> visit ( $r = 0.96$ ;  $p < 0.001$ ). GWG was determined by calculating the difference between the women's weight at the early pregnancy visit, and again at the mid-pregnancy visit. Maternal weight gain was then categorised based on the IOM GWG guidelines (**Table 1.3**). The GWG was presented quantitatively, as the total weight gain from early to mid-pregnancy, as well as categorically, as excessive versus non-excessive weight gain, again based on the IOM GWG guidelines (Rasmussen & Yaktine, 2009; Carreno et al., 2012; Brunner et al., 2015).

At both visits, the WHR was assessed, and was defined by the relationship between the waist circumference - the narrowest point between the lowest rib and the umbilicus, and hip circumference - the level of the greater trochanter, with the legs close together (Wang et al., 2003). Waist and hip circumference were assessed using a standardised measuring tape, with the subjects in an upright position and wearing lightweight clothing. MAC was measured at the centre between the topmost lateral point of the

acromion border, and the proximal and lateral border of the head of the radius. Measurements of the above circumferences were taken at eye level, and recorded to the nearest 0.1 cm.

The mark for both biceps (just above the measuring tape) and triceps (just below the measuring tape) were used to measure skinfold thickness (Marfell-Jones et al., 2006). Meanwhile, body fat percentages were measured from subcutaneous skinfolds (triceps, biceps, suprailiac, and subscapular), using a Harpenden caliper (British Indicators, Sussex, England), according to the procedure and equations identified by Durnin and Womersley (1974). The skinfold thickness equation has previously been used in many studies on pregnant women (Reynolds et al., 2010; Pike et al., 2013). Changes in body fat ( $\Delta$  body fat) were calculated from the differences in quantity of fat between early and mid-pregnancy.

Blood pressure (mm of Hg) was measured using a mercury sphygmomanometer, while the patient was relaxed and seated. Per the criteria set out by the International Society for the Study of Hypertension in Pregnancy, gestational HTN was identified as BP  $\geq$  140/90 mmHg (Redman & Russell, 2010). Moreover, metabolic syndrome (yes/no) was classified according to the guidelines provided by the USA National Heart, Lung and Blood Institute/American Heart Association (NHLBI/AHA) (Grundy et al., 2005), but with modifications suitable for the present research sample. Thus, abdominal circumference was not included as an indicator of obesity. Metabolic syndrome was distinct, with the occurrence of three or more of the following risk factors: BMI prior to pregnancy, with  $> 30 \text{ kg/m}^2$ ; TG  $\geq 1.7 \text{ mmol/L}$ ; HDL-cholesterol  $< 1.3 \text{ mmol/L}$ ; BP  $\geq 130/85 \text{ mmHg}$ , and fasting blood glucose  $\geq 8.3 \text{ mmol/L}$ .

## **2.5.2 Interview and Physical Activity Questionnaire**

The interview questionnaire was adapted from a previously published epidemiological survey in Saudi Arabia that included questions about socio-economic details, past medical and treatment history, and sun exposure (**Appendix III**) (Al-Daghri et al., 2011; Al-Musharaf et al., 2012; Al-Othman et al., 2012a; Aldesi, 2014). At the early



pregnancy visit, all socio-economic and clinical measurements were recorded, including the visit date, age of subject, place of birth, gestational age, maternal educational status, income, occupation, and area of residence. Clinical data included a family medical history, such as the existence of any chronic diseases, age of menarche, regularity of menstrual cycle, age at first pregnancy, date of LMP, and estimated date of delivery (EDD). Furthermore, as with previous studies (Wuertz et al., 2013; Choi et al., 2015; Ates et al., 2016; Bärebring et al., 2016; Lundqvist et al., 2016), the participants were asked about risk factors, such as parity (nulliparous or  $\geq 1$  multiparous). In addition, they were questioned about previous miscarriage (yes/no), number of caesarean sections, and previous pre-eclampsia, along with a historical list of medication, namely any anti-hyperlipidaemia and cardiovascular drugs used. The season of the sampling was recorded at both visits. For an analysis of season, in relation to vitamin D status, the months of the year were divided into two periods: April to October was classified as summer, and November to March as winter (Al-Daghri et al., 2012). The maximum UVB radiation in KSA occurs in July and the minimum values occur in December (Mahfoodh et al., 2003).

At both visits, the participants were asked about pregnancy-related symptoms, such as nausea, vomiting, morning sickness, tender/swollen breasts, frequent urination, headaches, mood swings, abdominal bloating, constipation, acute respiratory or gastrointestinal viral or bacterial infections, any hospitalisation or medication prescribed, and the presence of any pregnancy-related complications. Furthermore, they were asked about sun exposure at both visits, including sun exposure (yes/no) and other sun exposure indices, such as exposure to the sun at work (indoor or outdoor work), timing of the exposure (noon alone, or with either sunrise or sunset vs. just sunset or sunrise), clothing (whole body coverage vs. some parts of the body exposed), and the use of sunscreen (yes/no) (**Appendix III**). Noon time was defined in this study from 10 am to 2:00 pm.

Physical activity was also assessed at both visits, with a well-known and validated questionnaire being applied, namely an Arabic short version of an International Physical Activity Questionnaire (IPAQ) (Mohd et al., 2016). The short IPAQ has been

validated in various studies, among different populations, including pregnant subjects (Craig et al., 2003; Harrison et al., 2011; Takahasi et al., 2013). IPAQ evaluates physical activity during periods of leisure, work, and while commuting. It also assesses the intensity of activity as: vigorous, moderate, low-intensity physical activity/walking, or sitting. Each subject was assisted in recalling her physical activities over the previous week. For each type of activity, frequency and duration were recorded in days per week, and minutes or hours per day, respectively. This study excluded moderate and high activity because few women carried out such activities during pregnancy. The present study analysed low-intensity physical activity and minutes spent sitting per week, similar to other studies using IPAQ in pregnancy (Sukumar et al., 2015). Low-intensity physical activity in minutes per week was applied as a continuous and categorical variable, at less than or over 210 minutes/week (Mudd et al., 2013).

### **2.5.3 Dietary Data Collection and Processing**

The dietary tool applied for measuring the participants' food intake was the FFQ (**Appendix III**), and has been used previously in Saudi Arabian studies (Al-Musharaf et al., 2012; Al-Othman et al., 2012a; Al-Othman et al, 2012b). The subjects were interviewed separately, and the data gathered by applying a pre-designed questionnaire to measure aspects of food consumption among the expectant mothers, over the course of one week. The main purpose of this was to assess calcium, vitamin D, fat, and protein intake, as well as any other nutrients that may affect the absorption or excretion of vitamin D and calcium. Responses to the dietary questionnaire were facilitated through food models, various sized cups and spoons, cans and approximate portions, using hand gestures to help the participants recall the amount of food consumed.

The FFQ consisted of eight parts, pertaining to (1) bran, starch, and grains; (2) meat and fish; (3) fats and oils; (4) dairy products; (5) fruits and vegetables; (6) traditional dishes; (7) sweets and soft drinks; and (8) coffee and tea. Starches and grains mainly referred to any bran food that could have reduced calcium absorption (Weaver et al., 1999; Murray et al., 2000). Fruits and vegetables included those high in calcium, and any that may reduce calcium absorption (Heaney et al., 1988). Dairy fats (whole fat,

low fat, skimmed milk, or other products), and fat added during cooking may also affect calcium absorption (Heaney et al., 1995). Finally, data on supplement intake (yes/no) were collected, including multivitamins, folic acid, iron, vitamin D, and cod liver oil.

Nutrient intake was calculated using U.S. Department of Agriculture (USDA) software (27th edn, 2014), along with Nutribase software (11th edn, 2014), which utilises food macro- and micro-nutrient composition. For traditional Saudi food, an Arabic food analysis programme was used (1<sup>st</sup> version, 2007). The assessment of everyday food intake was conducted as total intake of macro- and micro-nutrients, especially vitamin D and calcium. Dietary nutrient values were compared with the Dietary reference intake (DRI) of micro-nutrients during pregnancy, such as vitamin D and calcium (IOM, 2010). Additionally, vitamin D and calcium intake were presented continuously and categorically as above 600 IU/day and 1000 mg/day of vitamin D and calcium, respectively.

## **2.6 Biochemical Assessment**

Blood samples (10 ml) were collected using sterile vacutainer blood collection apparatus. Whole blood, serum, and ethylene diamine tetra-acetic acid (EDTA) plasma were collected from the participants. All samples were aliquoted, and stored in a freezer at -80°C to facilitate their availability for subsequent chemical analysis. All the lab tests were performed on the serum, except haemoglobin A1c (HbA1c), which was performed on whole blood. Plasma was stored if needed for further analysis. The samples were stored and analysed in the BRP laboratory.

At the early pregnancy visit, random blood samples were collected to measure total 25(OH)D, serum glucose, insulin, HbA1c, lipid profile, calcium, phosphorus, albumin, creatinine, and alkaline phosphatase. At the mid-pregnancy visit, women were fasting and each participant underwent OGTT. 1-hour and 2-hour fasting blood samples were withdrawn to assess fasting glucose and insulin levels, 1- and 2-hour glucose, 2-hour insulin, homeostatic model assessment of insulin resistance (HOMA-IR) and beta cell function (HOMA- $\beta$ ). Higher HOMA-IR values indicated greater insulin resistance,

while lower HOMA- $\beta$  values indicated greater beta-cell dysfunction, as validated against gold standards (Herzberg-Schafer et al., 2010; Imamura et al., 2013). The assessments of other biochemical parameters measured at the 1<sup>st</sup> visit were also repeated.

### **2.6.1 Laboratory Techniques**

Total 25(OH)D and insulin were measured from the serum, using the Cobas e411 system (Roche Diagnostics GmbH, Mannheim, Germany). Serum albumin, creatinine, calcium, phosphorous, lipids, and glucose were measured using a routine laboratory chemical analyser (Konelab, Finland). Meanwhile, HbA1c was evaluated using the DCA Vantage Analyzer (Siemens Healthcare, Germany), and bone-specific alkaline phosphatase (BAP) was measured using the LIAISON XL immunoassay (DiaSorin, Italy).

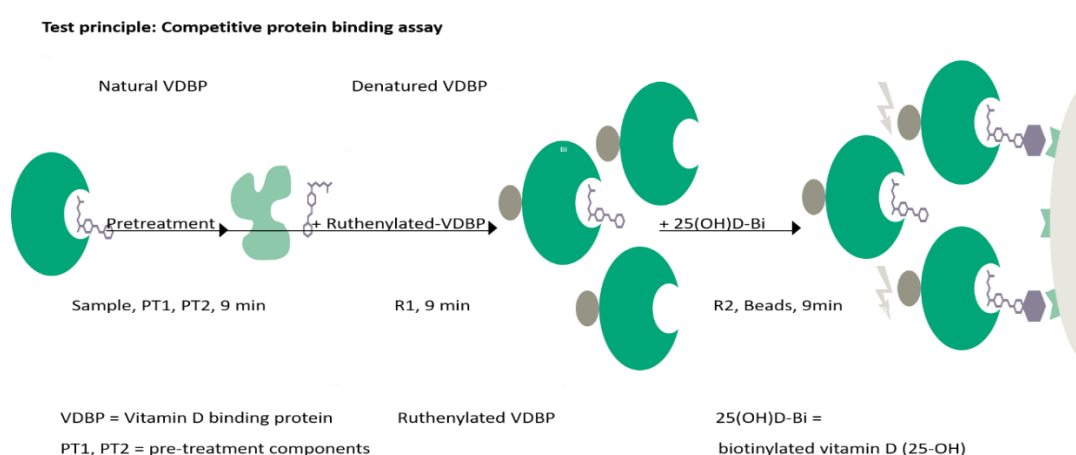
### **2.6.2 Vitamin D**

Serum levels of total 25(OH)D were measured using the electrochemiluminescence binding assay (ECLIA). The repeatability and intermediate coefficients of variation (CV) for the 25(OH)D assay were 4.4% and 6.6%, respectively, with 100% cross-reactivity to 25(OH) D<sub>3</sub>, and 92% cross-reactivity to 25(OH) D<sub>2</sub>. The measurement range between 7.50-175 nmol/L and any haemolysis samples were excluded. The BRP laboratory is a participating entity in the Vitamin D External Quality Assessment Scheme (DEQAS). Since no international consensus exists for vitamin D deficiency cut-off points during pregnancy, vitamin D was categorised according to 25(OH)D concentrations, as follows: < 25 nmol/L was considered to be a severe deficiency; 25-49.9 nmol/L was considered to be a deficiency; 50-74.9 nmol/L was considered as insufficiency, and  $\geq$  75 nmol/L was considered as sufficiency (Ross et al., 2011; Flood-Nichols et al., 2015).

#### **2.6.2.1 Test Principle**

The test principle used was a competitive protein binding assay test principle, with a total duration of 27 minutes. The vitamin D total assay employs vitamin D binding

protein to hold both 25(OH) D<sub>3</sub> and D<sub>2</sub>. This assay is directed towards the continuous determination of total 25(OH)D in human serum and plasma, to assist with the measurement of vitamin D adequacy. **Figure 2.3** describes the steps taken on the Cobas e411 platform, to measure vitamin D in the total assay, according to the manufacturer's protocol (adapted from Roche Diagnostics, 2012). The results were identified from an instrument-specific calibration curve, generated by 2-point calibration and a calibration master curve, provided by the reagent barcode.



**Figure 2.3** Vitamin D total assay (Roche Cobas ® e411). The procedure was conducted as follows: first, the sample was incubated with a pre-treatment reagent for nine minutes. The natural vitamin D binding protein in the sample was thus denatured to release the bound vitamin 25(OH)D. Second, the sample was further incubated with recombinant ruthenium-labelled vitamin D binding protein, to form a complex of vitamin 25(OH)D and the ruthenylated-vitamin D binding protein. Third, with the addition of biotinylated vitamin 25(OH)D, a complex consisting of the ruthenium-labelled vitamin D binding protein and biotinylated vitamin 25(OH)D was formed. The entire complex became bound to the solid phase (through the interaction of biotin and streptavidin-coated micro-particles, which were captured on the surface of the electrode). Unbound substances were removed. Voltage was applied to the electrode to induce chemiluminescent emission, measured with a photomultiplier.

## 2.6.3 Insulin, HOMA-IR and HOMA- $\beta$ Measurements

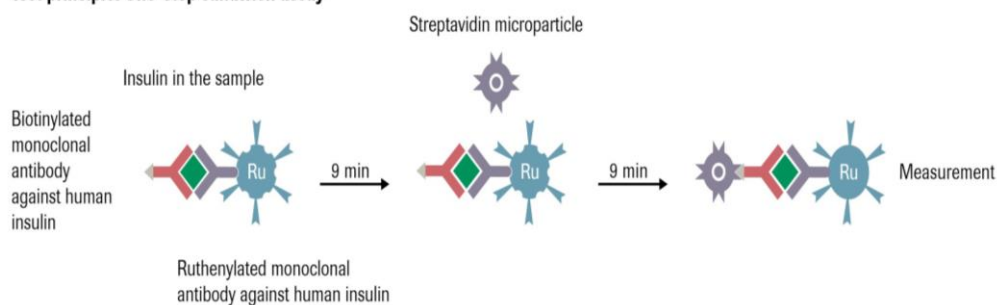
### 2.6.3.1 Insulin Determination

Serum and free-insulin concentrations were measured using electrochemiluminescence (ECL) assay. The repeatability and intermediate CV for the insulin assay was 2.0% and 2.6%, respectively, with a test measurement range of between 0.2 and 1000  $\mu\text{U/mL}$ .

### 2.6.3.2 Test Principle

The test principle used, was a one-step sandwich assay test principle, with a total duration of 18 minutes. **Figure 2.4** demonstrates the procedure for the insulin ECL assay. The results were identified through a calibration curve, which was instrument-specifically created using a 2-point calibration and master curve, delivered via the reagent barcode.

#### Test principle: One-step sandwich assay



#### Step 1 (9 minutes):

20  $\mu\text{L}$  of the patient sample are incubated with a biotinylated monoclonal antibody and a ruthenylated monoclonal antibody, both against different epitopes on human insulin. A sandwich immune-complex is formed with insulin carrying a biotinylated and a ruthenylated antibody.

#### Step 2 (9 minutes):

After the addition of streptavidin-coated paramagnetic microparticles, the sandwich complexes bind to the solid phase via biotin-streptavidin.

#### Step 3 (measurement):

The reagent mixture is transferred to the measuring cell, where the microparticles are fixed to the electrode surface by magnetic action. The unbound substances are subsequently removed by ProCell. Luminescence is then induced by applying a voltage and measured with a photomultiplier.

**Figure 2.4** Insulin ECL assay test principle (adapted from Roche Diagnostics, 2011). The procedure is described in the steps shown in the above Figure.

### **2.6.3.3 HOMA-IR and HOMA- $\beta$**

The homoeostasis model of insulin resistance and  $\beta$ -cell function was calculated using HOMA-IR and HOMA- $\beta$  Matthews equations, respectively (Matthews et al., 1985; Wallace et al., 2004). This was calculated as follows:

$$\text{HOMA-IR} = (\text{Fasting insulin } \mu\text{U/mL} \times \text{Fasting glucose mmol/L})/22.5$$

$$\text{HOMA-}\beta = (20 \times \text{Fasting insulin } \mu\text{U/mL})/(\text{Fasting glucose mmol/L}-3.5)$$

### **2.6.4 HbA1c**

To measure the level of HbA1c in whole blood, a DCA Vantage Analyzer point-of-care (POC) device was used, and was based on the latex immune agglutination method. Blood glucose adheres to haemoglobin in red blood cells, to create glycosylated haemoglobin, called haemoglobin A1c or HbA1c. HbA1c provides a long-term measurement of blood glucose levels in diabetic patients, indicating blood glucose control over the preceding three-month period. A drop of blood from the sample was applied to a sample cartridge, which was then analysed over a period of five minutes in a desktop analyser, to measure the percentage of HbA1c. In addition, latex agglutination was inhibited, which is a synthetic polymer containing multiple copies of the immunoreactive portion of haemoglobin A1c. The latter causes the agglutination of latex, coated with haemoglobin A1c-specific murine monoclonal antibody. This then leads to increased light-scattering, measured as a rise in absorption at 531 nm. Haemoglobin A1c in whole blood samples compete for binding sites, which are limited in number, due to the inhibition of agglutination and decreased light-scattering, measured as a decrease in absorption at 531 nm.

### **2.6.5 Serum Glucose, Lipid, Calcium, Creatinine, Albumin, and Phosphorous Assays**

Serum glucose, lipid profile (HDL-cholesterol, total cholesterol, and triglyceride), calcium, creatinine, albumin, and phosphorous were measured in a routine laboratory analysis (Konelab, Finland). Calibration of the biochemical analyser, for all serum samples, was regularly performed prior to the analysis using quality control samples, provided by the manufacturers (ThermoFisher Scientific, Espoo, Finland).

To assess blood levels of glucose concentration in human serum, the Konelab method was used. This employs glucose oxidase and a modified Trinder colour reaction, catalysed by the enzyme, peroxidase. Glucose was oxidised to D-gluconate through glucose oxidase, with the formation of an equimolar amount of hydrogen peroxide. In the presence of peroxidase, 4-aminoantipyrine and phenol were oxidatively coupled by hydrogen peroxide to form a quinonimine dye that was red in colour. The intensity of the colour in the reaction was measured at 510 nm, and this was proportionate to the glucose concentration in the sample.

In addition, triglycerides were hydrolysed by the lipoprotein lipase to glycerol and fatty-acids. The glycerol was phosphorylated to glycerol-3-phosphate by glycerol kinase, which was then oxidised to dihydroxyacetone phosphate and hydrogen peroxide, mediated by glycerol-3-phosphateoxidase. The hydrogen peroxide reacted with 4-aminoantipyrine and 4-chlorophenol to form a quinoneimine dye, mediated by phosphate oxide. The absorption of the colour formed, was measured at 510 nm and the results were automatically calculated using the Konelab Analyser in a calibration curve. A triglyceride level  $\geq 1.7$  mmol/L was considered abnormal (Grundy et al., 2005).

Cholesterol esters were enzymatically hydrolysed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including whatever was originally present, was then oxidised by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. Mediated by peroxidase, the hydrogen peroxide combined with hydroxybenzoic acid and 4-aminoantipyrine to form a chromophore (quinoneimine dye), which could be quantitated at 500-550 nm (Allain et al., 1974).

The HDL-cholesterol assay was finally undertaken as a homogeneous enzymatic colorimetric test in the presence of magnesium sulphate, dextran sulphate, which selectively forms water-soluble complexes with LDL, very low-density lipoprotein (VLDL), and chylomicrons, which are resistant to polyethylene glycol (PEG)-modified enzymes. The cholesterol concentration of HDL-cholesterol was enzymatically determined by cholesterol oxidase, coupled with PEG to the amino groups (approximately 40%). The results were automatically calculated by the Konelab



Analyser, using a calibration curve. Low HDL-cholesterol was subsequently defined as < 1.03 mmol/L (Grundy et al., 2005; Lv et al., 2016), and the abnormal total cholesterol-HDL ratio was defined as > 3.5 (Kannel, 1983). LDL-cholesterol and the total cholesterol-to-HDL ratio were calculated using the following equations (Friedewald et al., 1972):

$$\text{LDL} = \text{Total Cholesterol} - \text{HDL} - (0.16 \times \text{Triglyceride})$$

$$\text{Total Cholesterol/HDL Ratio} = \text{Total Cholesterol} / \text{HDL Cholesterol}$$

The albumin assay test principle was based on an albumin reaction with a specific dye, bromocresol purple, to form a coloured product. This test was grounded on the measurement of the colour intensity developed at 600 nm (Pinnell & Northam, 1978; Parviainen et al., 1985). Conversely, A serum calcium assay test principle was applied using electrolyte measurements in Konelab Analysers, carried out directly with ion-selective electrodes, and without any dilution of the sample. Corrected calcium concentration (mmol/L) was calculated from the following formula (Kanis & Yates, 1985):

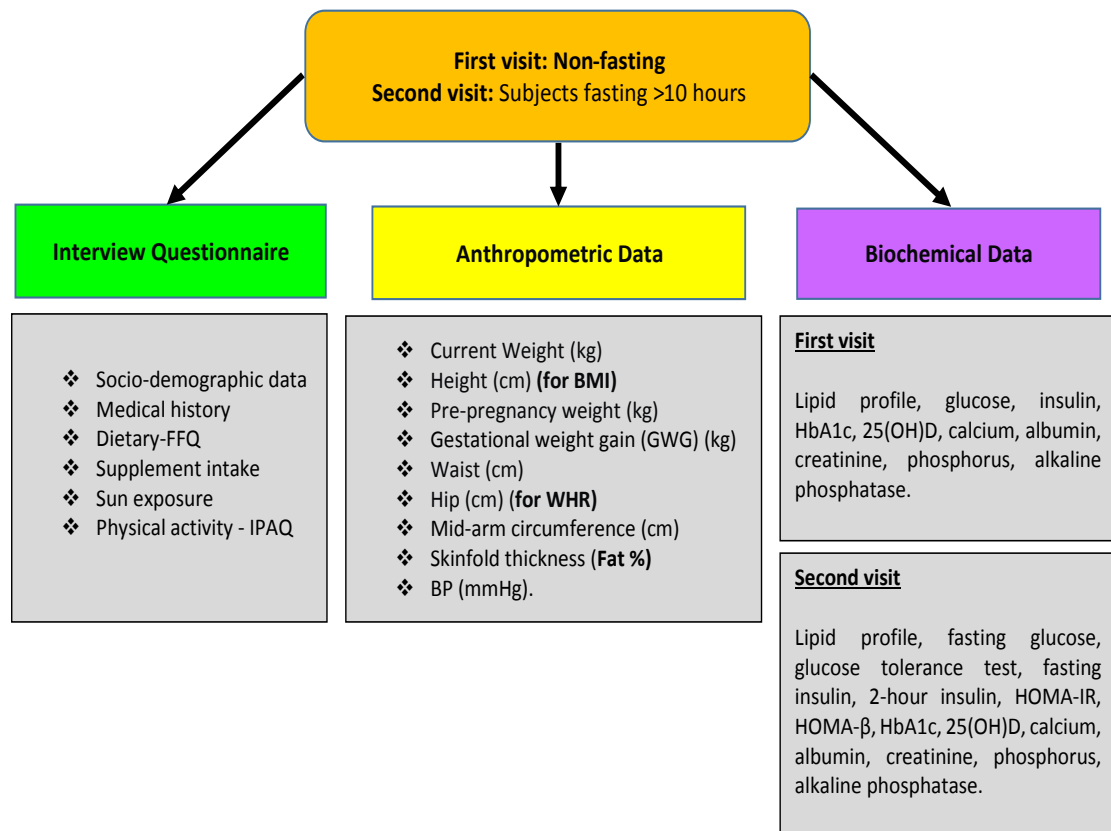
$$\text{Corrected calcium in mmol/L} = (\text{Calcium in mmol/L} / 0.25) + 0.8 \times (4 - \text{Albumin in gm/L} / 10) \times 0.25$$

The principle of the phosphorus assay is based on the fact that phosphate forms in the acidic medium of a yellow-coloured complex with ammonium molybdate. The intensity of this colour was proportionate to the concentration of inorganic phosphate in the sample and was measured at 340 nm (Daly & Ertingshausen, 1972).

Meanwhile, the creatinine assay test principle was converted to sarcosine, with the aid of creatininase and creatinase. Sarcosine was then converted to glycine, formaldehyde, and hydrogen peroxide in the presence of oxygen, using sarcosine oxidase. The liberated hydrogen peroxide reacted with 4-aminophenazone and 2, 4, 6-triiodo-3-hydroxybenzoic acid to form a quinoneimine chromogen in a reaction catalysed by peroxidase. The colour intensity was directly proportionate to the concentration of creatinine present, and could be measured photometrically at 540 nm.

### 2.6.6 The Bone-Specific Alkaline Phosphatase Assay (BAP)

Bone-specific alkaline phosphatase was measured using the LIAISON XL (DiaSorin, Italy) immunoassay, which is a direct two-site sandwich assay, utilising two affinity-purified mouse monoclonal antibodies for the capture and detection of alkaline phosphatase.



**Figure 2.5** Summary of data collection at both visits. Data collected in the study included an interview questionnaire, anthropometric measurements and biochemical parameter.

## 2.7 Data Analysis

Data were analysed using SPSS version 21.0 statistical software (SPSS, Chicago, IL, USA). The normality of all quantitative variables was tested before performing the analysis, using Shapiro-Wilk Test. Quantitative normally distributed variables were

reported using mean and standard deviations, while quantitative non-normally distributed variables were reported using medians (1<sup>st</sup> and 3<sup>rd</sup> quartiles). Frequencies and percentages were applied in the reporting of categorical variables. For subsequent meaningful statistical analysis, 25(OH)D was categorised using the common clinical cut-off of deficient (25(OH)D < 50 nmol/L) and adequate (25(OH)D ≥ 50 nmol/L) levels, based on the recommendations of the Endocrine Society (Farrant et al., 2008; Dawson-Hughes et al., 2010; Holick et al., 2011; Cho et al., 2013; Parildar et al., 2013; Wuertz et al., 2013). Pearson's chi-square test was used to determine the association between categorical variables, while group differences were distinguished via an independent sample t-test and Mann-Whitney U test. These tests were conducted for normally and non-normally distributed variables, respectively. Adjustment for age and BMI as covariates between the groups was carried out through analysis of covariance (ANCOVA).

The Wilcoxon Signed Rank Test was used to compare median vitamin D levels from early to mid-pregnancy, and adjustments were made for mid-pregnancy gestational age, multivitamin intake, physical activity, estimated vitamin D intake, sun exposure, and season, using repeated measures ANCOVA. The difference across two visits was evaluated using a paired sample t-test and Wilcoxon signed rank test for normally and non-normally distributed variables, respectively. Analysis of variance (ANOVA) was then undertaken to compare normally distributed variables, categorised into more than two groups. For non-normally distributed data, an independent sample Kruskal Wallis Test was applied.

Finally, the Pearson's and Spearman's rank correlation coefficients were determined to assess the linear relationship between quantitative variables for normally and non-normally distributed variables, respectively. Logistical regression was used to identify the risk factors associated with categorical outcome variables. A p-value of < 0.05 and 95% CI was finally applied to report statistical significance and the precision of the estimates.

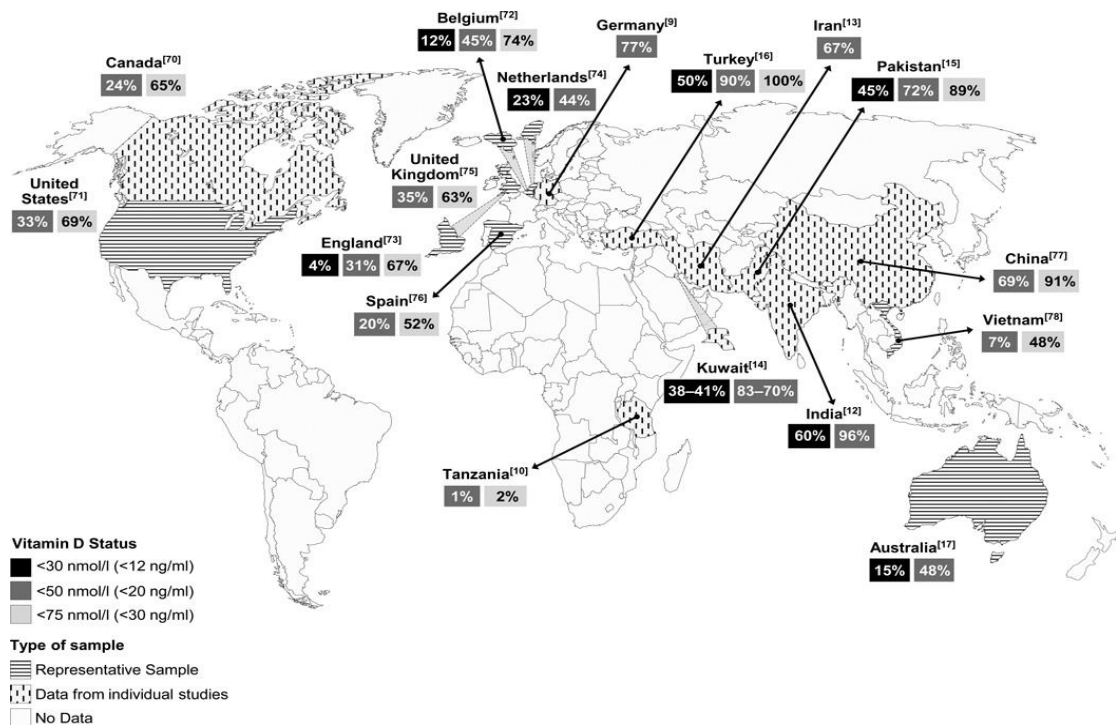
## **Chapter 3**

### **Prevalence of Vitamin D Deficiency in Early Pregnancy and its Predictors**

### 3.1 Introduction

The main sources of vitamin D are either endogenous production or from the diet. The ubiquitous distribution of vitamin D receptors on a wide range of tissues, indicates multi-faceted and crucial role of this vitamin (John et al., 2007). 25(OH)D deficiency appears to be a global health hazard with approximately one billion sufferers all over the world Holick and Chen (2008). Hypovitaminosis D has been implicated in a wide variety of conditions such as osteoporosis, rickets, cardiovascular diseases, autoimmune diseases, cancer, type 2 diabetes mellitus, and adverse maternal and foetal outcomes (Aghajafari et al., 2013).

Between 80-100% of Saudi women during their reproductive phase of life have suboptimal levels of vitamin D (Al-Mogbel, 2012; Hussain et al., 2014; Fouda et al., 2016). Higher prevalence of vitamin D deficiency among Saudi women appears to be a predisposition for further deterioration of vitamin D status particularly among pregnant females because of the increased nutritional demands of growing foetus (Urrutia & Thorp, 2012). This is evident from the fact that in Saudi Arabia, Kuwait, the UAE, and Iran, 10-80% of mothers and 40-80% of their neonates at the time of delivery have been shown to have suboptimal serum levels of 25(OH)D (Molla et al., 2005; Ainy et al., 2006; Kazemi et al., 2009; Aly et al., 2013). Nevertheless, the rate of hypovitaminosis D varies, depending on the location, ethnicity, assay used for assessment, and definition of deficiency itself (**Figure 3.1** shows the prevalence of vitamin D deficiency worldwide).



**Figure 3.1** Prevalence of hypovitaminosis D in pregnant or lactating women, worldwide (adapted from Palacios & Gonzalez, 2014).

Aetiology of hypovitaminosis D during pregnancy is multi-factorial. Factors and limited exposure to the sunlight, less vitamin D and calcium intake, ethnicity, age, socio-economic status, cigarette smoking, alcohol intake, repeated pregnancies, obesity, fat malabsorption syndrome, medication, increased vitamin D catabolism, and chronic liver or kidney disorders have been implicated in hypovitaminosis D (Holick & Chen, 2008; Andersen et al., 2013; Flood-Nichols et al., 2015; Karras et al., 2016). Additionally, genetic polymorphisms and gene mutations, such as the CYP2R1 mutation, may aggravate vitamin D deficiency (Touvier et al., 2015; Thacher & Levine, 2016). Furthermore, an increase in nutritional demands during pregnancy is a major factor contributing to vitamin D deficiency, so pregnancy itself can be a potential risk factor (Garabedian & Ben-Mekhbi, 1999; Holmes et al., 2009; Choi et al., 2015).

Vitamin D deficiency during pregnancy has been investigated in a limited number of studies on a relatively smaller number of participants in the Gulf states (Molla et al., 2005; Al Kalbani et al., 2011; Al-Faris, 2016).

To date, almost all the studies assessing vitamin D deficiency during pregnancy in the Gulf states have been performed on a relatively small number of participants (Molla et al., 2005; Al Kalbani et al., 2011; Al-Faris, 2016). Moreover, the vast majority of these studies assessed 25(OH)D status in the latter stages of pregnancy, but failed to investigate lifestyle factors. However, an assessment of suboptimal vitamin D levels in the initial stages of pregnancy offers an opportunity for early detection, which may be critical for avoiding adverse maternal and foetal complications. The aim of the present study is, therefore, to assess the prevalence of vitamin D deficiency during early pregnancy, and to identify factors predisposing pregnant women to such deficiency.

## **3.2 Research Design and Methods**

### **3.2.1 Study Population**

A total of 578 Saudi women, with a mean age of  $28.8 \pm 5.4$  years (range 18-39 years), were recruited in their first trimester (8-12 weeks). Inclusion and exclusion criteria were given in Chapter 2.

### **3.2.2 Assessment of Circulating 25(OH)D and Biochemical Parameters**

Venous whole blood was collected from the cubital fossa of non-fasting participants (10 mL), to assess levels of 25(OH)D, calcium, phosphorus, creatinine, and alkaline phosphatase, along with blood glucose, insulin, and the lipid profile. Random serum glucose, calcium, creatinine, albumin, phosphorus, and lipid profiles (HDL-cholesterol, total cholesterol and triglyceride) were measured using routine laboratory analysis (Konelab, Finland). Alkaline phosphatase was measured using the LIAISON XL immunoassay (DiaSorin, Italy), while HbA1c was measured using the DCA Vantage analyzer (Siemens Healthcare, Germany). Total 25(OH)D and serum insulin were measured in an ECLIA and ECL assay, respectively (Cobas e 411; Roche Diagnostics GmbH, Mannheim, Germany). The cut-off points used were described in Chapter 2.

### **3.2.3 Determinants of Circulating 25(OH)D Levels**

Anthropometrics were collected as described in Chapter 2. Using a FFQ (**Appendix III**), quantitative aspects of food consumption over a period of seven days were recorded to assess calcium, vitamin D, and protein intake, along with any other nutrients that would possibly affect the absorption or excretion of vitamin D (Al-Musharaf et al., 2012; Al-Othman et al., 2012b). Nutrient intake was calculated using specialised programs as shown in Chapter 2. The pregnant subjects were then questioned about their dietary intake of multivitamins (yes/no). Moreover, physical activity was assessed by a short version of the Arabic IPAQ (Hernandez-Cordero et al., 2008; Bertolotto et al., 2010; Harizopoulou et al., 2010; Ebrahimi et al., 2015). Low-intensity physical activity/walking and sedentary time were determined using the guidelines for data



processing and IPAQ analysis (IPAQ Research Committee, 2005). Low-intensity physical activity in minutes per week was then presented as a continuous and categorical variable of equal or greater than, 210 minutes/week (Mudd et al., 2013).

### **3.2.4 Data Analysis**

The sample size was estimated for adequate statistical power. It was determined 246 pregnant women were required, assuming a prevalence of 80% vitamin D deficiency (Azhar, 2009), at a 5% level of significance and with  $\pm 5\%$  level of precision (width of 95% confidence interval). However, the study enrolled 578 pregnant women to account for possible attrition.

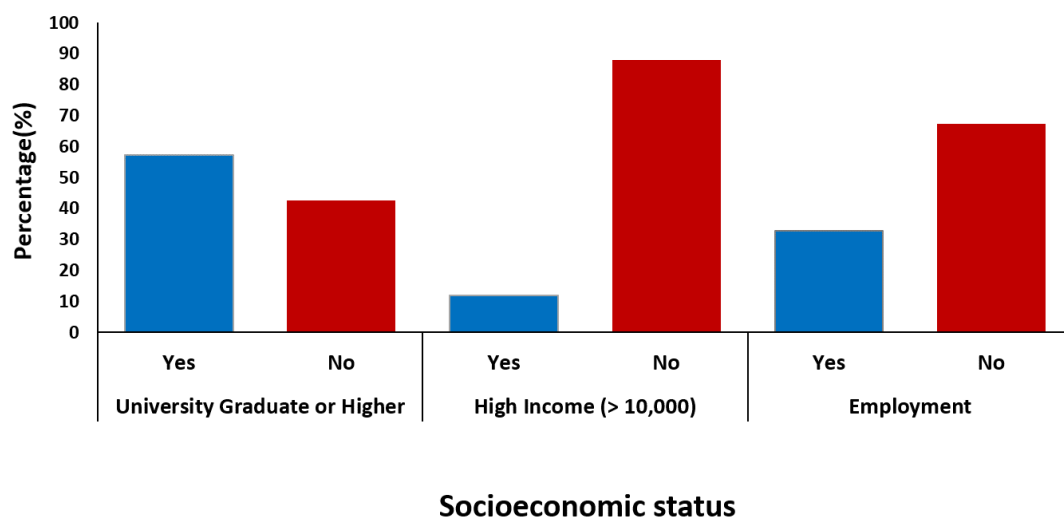
For subsequent meaningful statistical analysis, vitamin D levels were categorised into deficient ( $< 50$  nmol/L) and non-deficient groups ( $\geq 50$  nmol/L) (Farrant et al., 2008; Dawson-Hughes et al., 2010; Holick et al., 2011; Cho et al., 2013; Parildar et al., 2013; Wuertz et al., 2013). Pearson's chi-square test was applied to determine the association between the categorical variables (deficient vs. non-deficient). Comparisons between groups were then made through an independent sample t-test and the Mann-Whitney U test for normally and non-normally distributed variables, respectively. Adjustment for age and BMI as covariates between the groups were done using ANCOVA. Pearson's and Spearman's rank correlation coefficients were also determined to assess the linear relationship between quantitative variables for normally and non-normally distributed variables, respectively. Moreover, logistic regression enabled the outcome variables for risk factors associated with vitamin D deficiency to be identified. Two models were applied to adjust for possible confounders: Model 1 - Age, BMI, and sun exposure, and Model 2 - Model 1 + parity, season, vitamin D intake, multi-vitamin intake, physical activity, education, employment, living in North Riyadh, and extent of skin coverage with clothing. All three ORs were reported (unadjusted, adjusted for Model 1 and Model 2), and a p-value of  $< 0.05$  was considered statistically significant.

## 3.3 Results

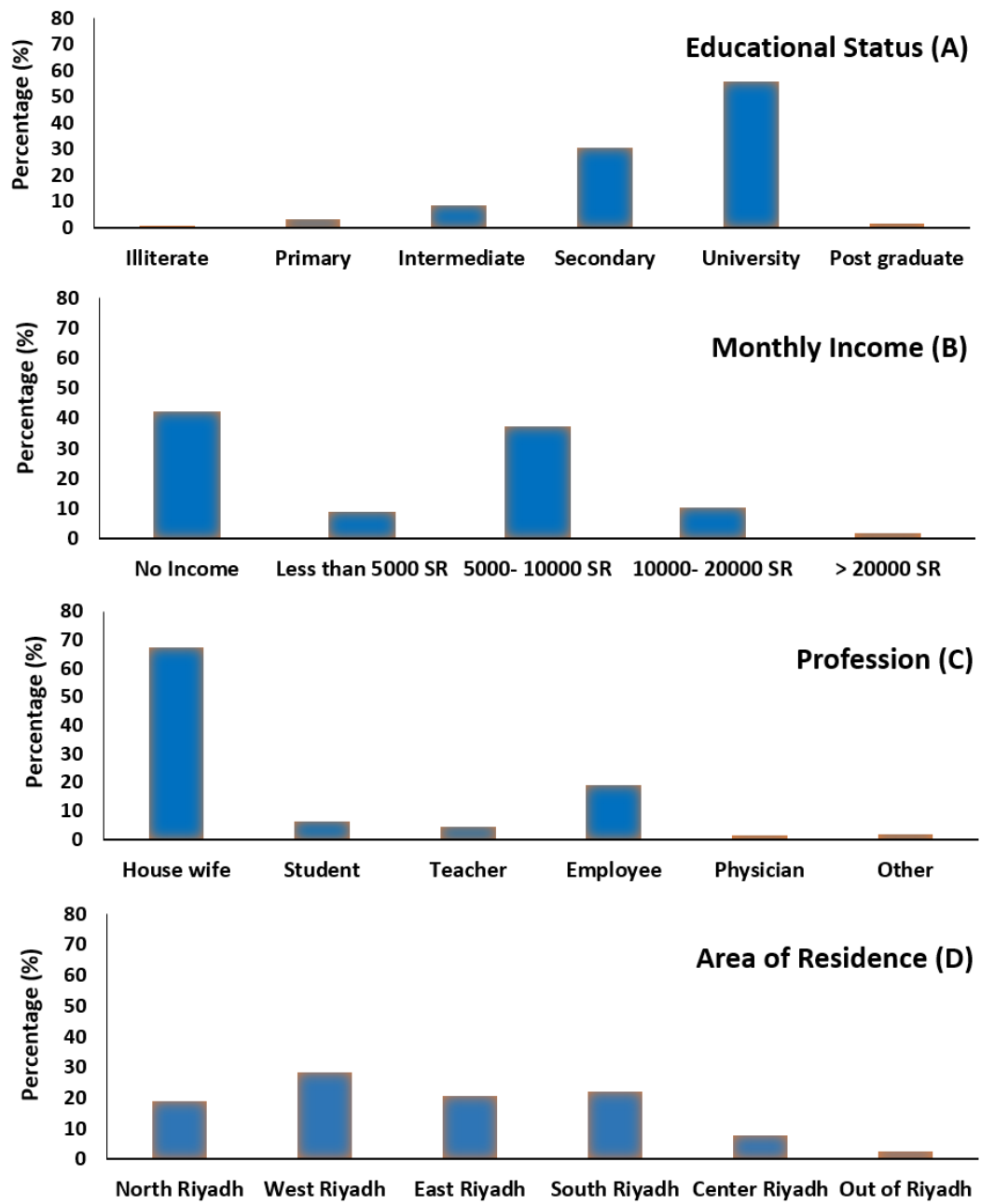
### 3.3.1 General Characteristics of Subjects During Early Pregnancy

#### 3.3.1.1 Socio-economic Status

As mentioned in Chapter 2, of the 773 pregnant women originally recruited, 578 attended the first visit of their gestation. The mean maternal age was  $28.8 \pm 5.4$  (range 18-39) years old. Of the total women in the study, 320 (57.3%) were university graduates or post-graduates, 183 (32.7%) were employed, and 62 (12%) earned an income >10,000 Saudi Riyals (**Figure 3.2**). The majority (28.4%) of these women lived in West Riyadh, with comparable percentages noted in the South (21.9%), East (20.5%) and North (19%) of the city (**Figure 3.3 D**). Educational status, monthly income, profession, and area of residence in Riyadh is presented for the participants in general (**Figure 3.3 A-D**).



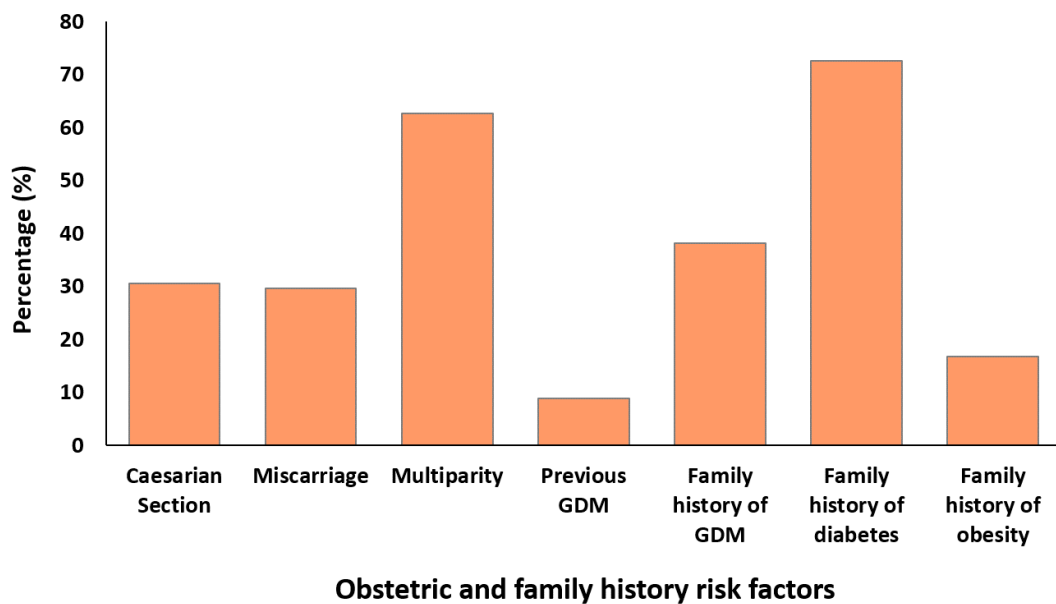
**Figure 3.2** Educational Status, monthly income and employment amongst the general participants. In the above figure, values are shown in percentages.



**Figure 3.3** (A) Education, (B) Monthly income, (C) Profession of the participants, and (D) Area of residence. In the above figure, values are shown in percentages.

### 3.3.1.2 Obstetric Data and Family History

Of the total women, 195 had a nulliparous pregnancy (37.3%), whereas 362 (62.7%) were multiparous, 129 (30.5%) had previously had a caesarean section, and 120 (29.6%) had experienced miscarriage. Thirty eight (8.8%) had experienced gestational diabetes and 164 (38.2%) had a family history of GDM, while 347 (72.6%) had a family history of diabetes (Figure 3.4 presents obstetric data on the women, as well as their family history).



**Figure 3.4** Obstetric and family history for the overall sample. Data presented as frequencies and percentages. Multiparity was defined  $\geq 1$  births. All other variable are categorical and presented as ‘yes/no’.

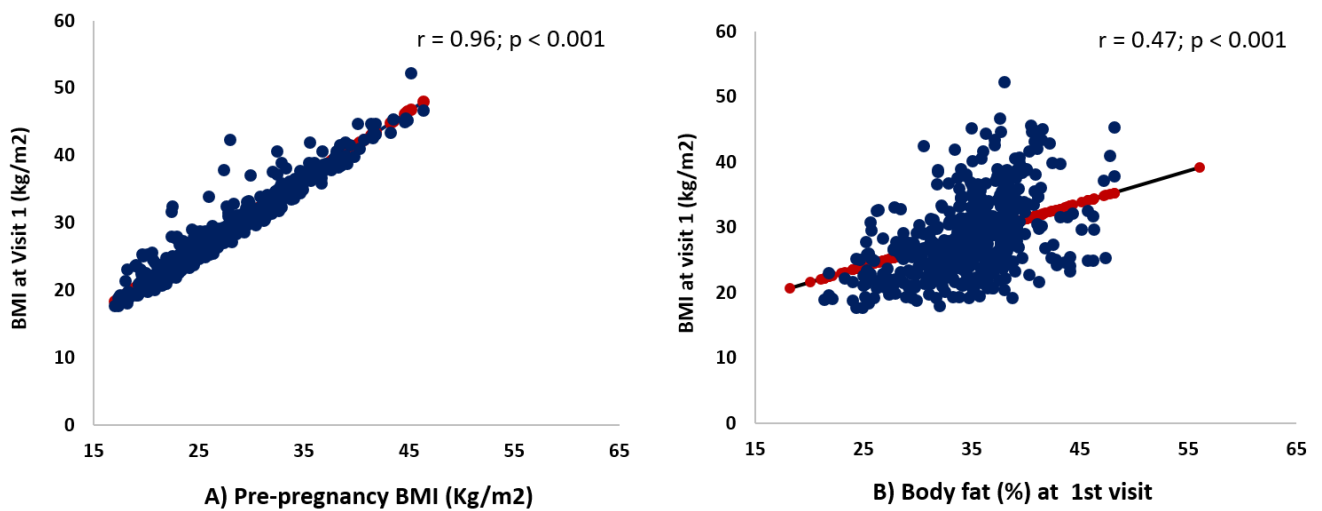
### ***3.3.1.3 Anthropometric and Biochemical Characteristics***

The anthropometric and biochemical characteristics of the study population are shown in **Table 3.1**. The mean pre-pregnancy BMI, and BMI in early pregnancy were  $27.0 \pm 6.0$  and  $28.0 \pm 6.3 \text{ kg/m}^2$ , respectively. In early pregnancy, 183 (31.9%) of the pregnant women were overweight, and 195 (34%) were obese. Additionally, pre-pregnancy BMI was strongly correlated with BMI during early pregnancy (**Figure 3.5 A**). In addition, BMI in early pregnancy was moderately correlated with percentage body fat (**Figure 3.5 B**). Pregnant women with high blood pressure numbered 23 (4.3%), and 279 (48.3%) in the cohort had low HDL-cholesterol  $< 1.03 \text{ mmol/L}$  (**Figure 3.6**).

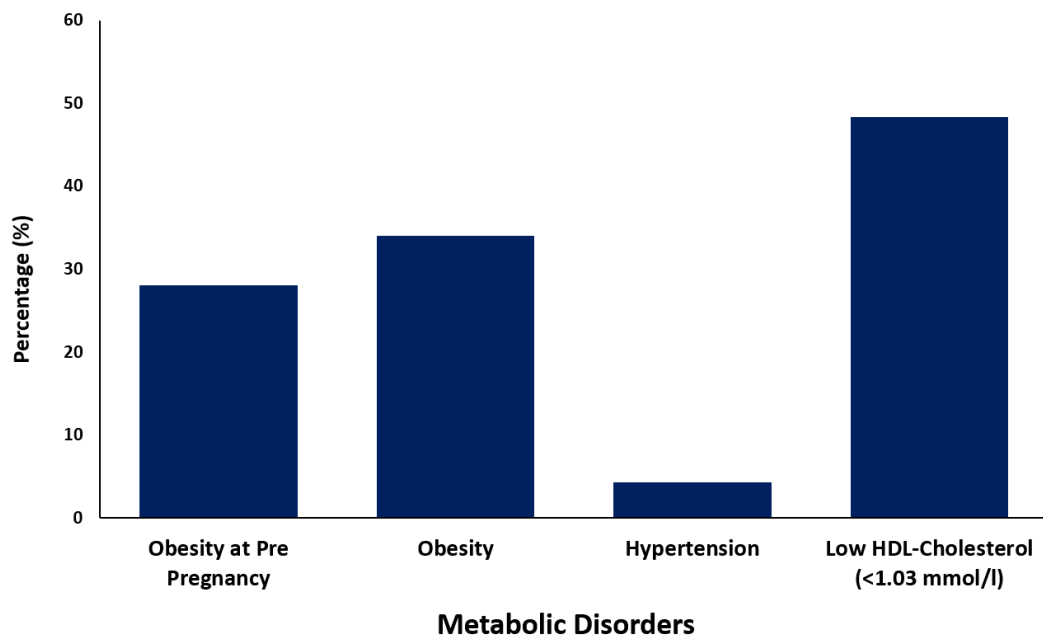
**Table 3.1 Anthropometric and biochemical characteristics during early pregnancy**

<b>Anthropometric Parameters</b>	<b>Total N = 578</b>
Age (Years)	28.8 ± 5.4
Gestational age (weeks)	12.0 ± 3.0
Pre-pregnancy BMI (kg/m <sup>2</sup> )	27.0 ± 6.0
BMI at 1 <sup>st</sup> visit (kg/m <sup>2</sup> )	28.0 ± 6.3
Waist circumference (cm)	91.5 ± 13.6
Hip circumference (cm)	107.9 ± 12.0
Waist-hip ratio	0.8 ± 0.1
Systolic BP (mmHg)	113.9 ± 12.7
Diastolic BP (mmHg)	67.9 ± 9.6
Body fat (%)	34.3 ± 5.6
<b>Biochemical Parameters</b>	
Vitamin D (nmol/l) #	28.7 (19.7 - 43.8)
Corrected calcium (mmol/l)	2.2 ± 0.2
Phosphorus (mmol/l)	1.2 ± 0.4
Alkaline phosphatase (mmol/l)	9.7 ± 3.3
Creatinine (μmol/l)	55.8 ± 18.2
Glucose (mmol/l) #	4.8 (4.4 - 5.3)
Insulin (uU/mL) #	8.5 (4.8 - 18.4)
HbA1c (%)	5.1 ± 0.5
Total cholesterol (mmol/l)	5.2 ± 1.0
HDL-cholesterol (mmol/l)	1.3 ± 0.3
Total cholesterol/HDL Ratio	4.0 ± 1.0
LDL-Cholesterol (mmol/l)	3.2 ± 0.8
Triglycerides (mmol/l)	1.4 ± 0.6

**Note:** Data presented as mean and standard deviation for normal variables, the median, first and third quartiles are presented for non-normally distributed variables. # Indicates non-normally distributed variables. The Assumption of Normality was tested using the Shapiro-Wilk Test.



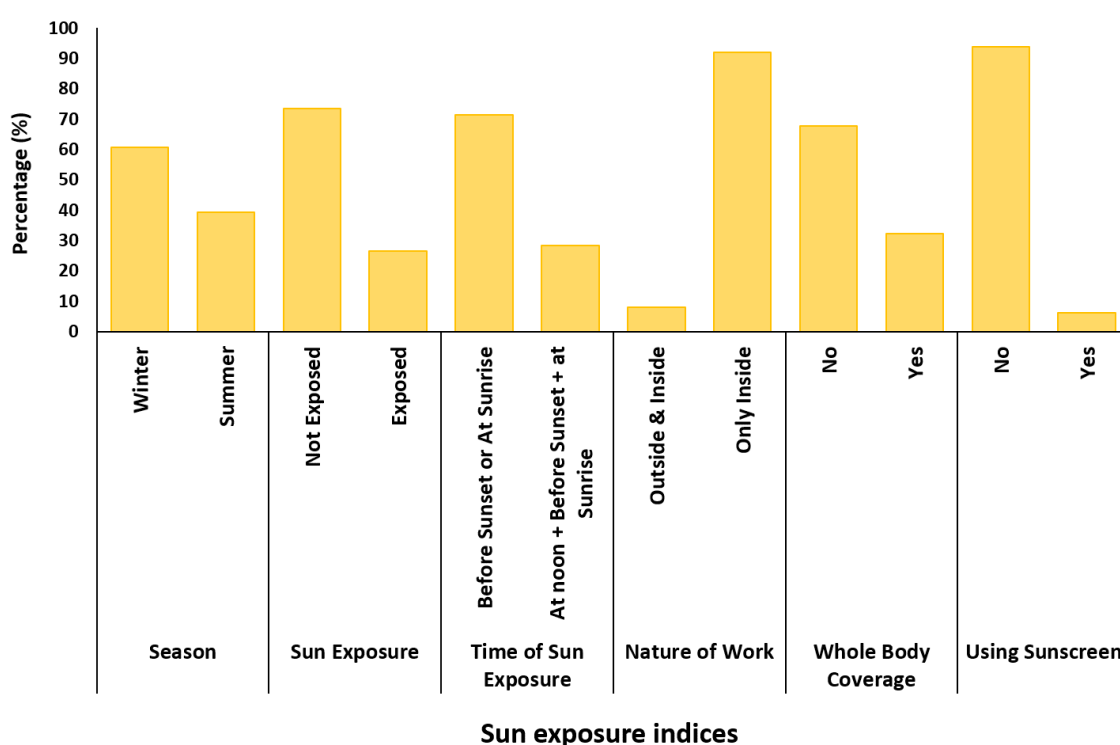
**Figure 3.5** Correlation of BMI during early pregnancy with (A) Pre-pregnancy BMI, and (B) Percentage body fat during early pregnancy; ‘r’ represents Pearson’s correlation coefficient.



**Figure 3.6** Presence of metabolic disorders during early pregnancy. The values show the percentage of women with different metabolic disorders in early pregnancy.

### 3.3.1.4 Lifestyle Factors

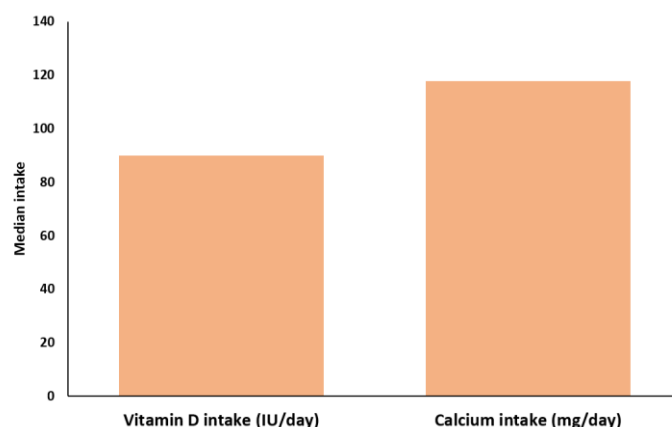
The percentage of blood samples collected from the pregnant women during the summer amounted to 39.4%, with 60.6% being collected in winter. Exposure to the sun was reported by 154 (26.6%) of the women (yes/no), while 165 (28.5%) were exposed to the sun noon time alone, plus either sunset or sunrise, or both. The number of women whose work took place exclusively indoors totalled 91.9% (n = 531). Regarding their clothing, 186 (32.2%) reported that their entire body was covered and only 6.2% of the participants used sunscreen lotion for protection (**Figure 3.7** displays general percentages of sun exposure indices in early pregnancy).



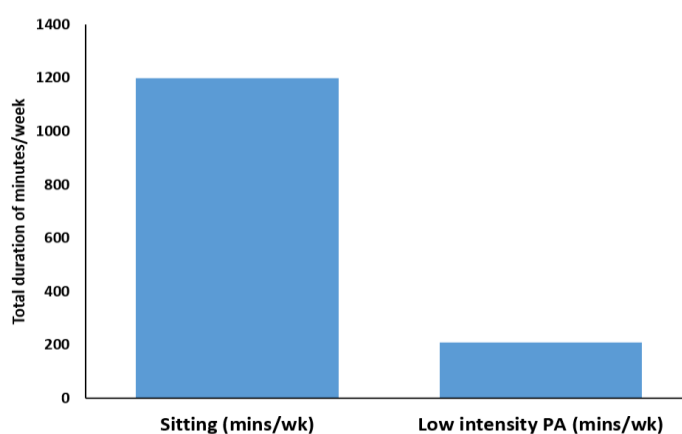
**Figure 3.7** Sun exposure indices in early pregnancy. The values are presented in percentages.



Estimated vitamin D and calcium intake amongst the women during early pregnancy was 89.9 IU/day (63.5-169.0) and 117.7 mg/day (60.2-370.3), respectively (**Figure 3.8**). These data indicate that in 2.6% of the cases, vitamin D intake was above the DRI, and in 4.5% of the cases, calcium intake was also above the DRI. A quarter (25%) of the women reported the use of multivitamin supplements in early pregnancy. Also used here was the IPAQ median for sedentary activity of 1,200 minutes/week (600-1800), with low intensity physical activity/walking for 210 minutes/week (70-600) (**Figure 3.9**).



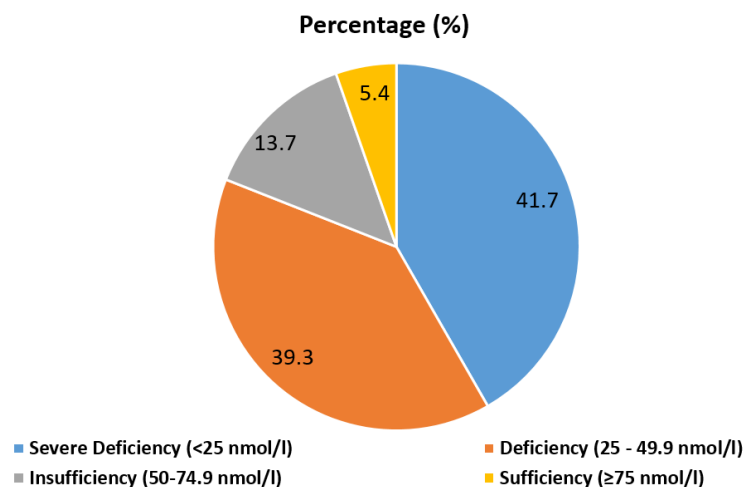
**Figure 3.8** Estimated vitamin D and calcium intake in early pregnancy. The values show the median intake of vitamin D and calcium from food.



**Figure 3.9** Physical activity (PA) in early pregnancy. The values show the median sedentary and physical activity in minutes per week.

### 3.3.2 Vitamin D Deficiency in Early Pregnancy

The median (interquartile range) 25(OH)D concentration in the pregnant women studied here was 28.7 nmol/L (19.7 - 43.8), which was considered to be deficient. A total of 41.7% (241/578) of participants were severely vitamin D deficient (< 25 nmol/L), while 39.3% (227/578) were deficient (25-49.9 nmol/L); 13.7% (79/578), insufficient (50-74.9 nmol/L), and 5.4% (31/578) presented with sufficient vitamin D levels ( $\geq 75$  nmol/L) (**Figure 3.10**). Therefore, only a relatively small number of the women ( $n = 31$ ) achieved sufficient vitamin D levels. The population sample was subsequently categorised into either deficient (< 50 nmol/L) or non-deficient ( $\geq 50$  nmol/L) groups for meaningful statistical analysis (Farrant et al., 2008; Dawson-Hughes et al., 2010; Holick et al., 2011; Cho et al., 2013; Parildar et al., 2013; Wuertz et al., 2013). The proportion of vitamin D deficiency (< 50 nmol/L) in early pregnancy amounted to 81% (468/578).



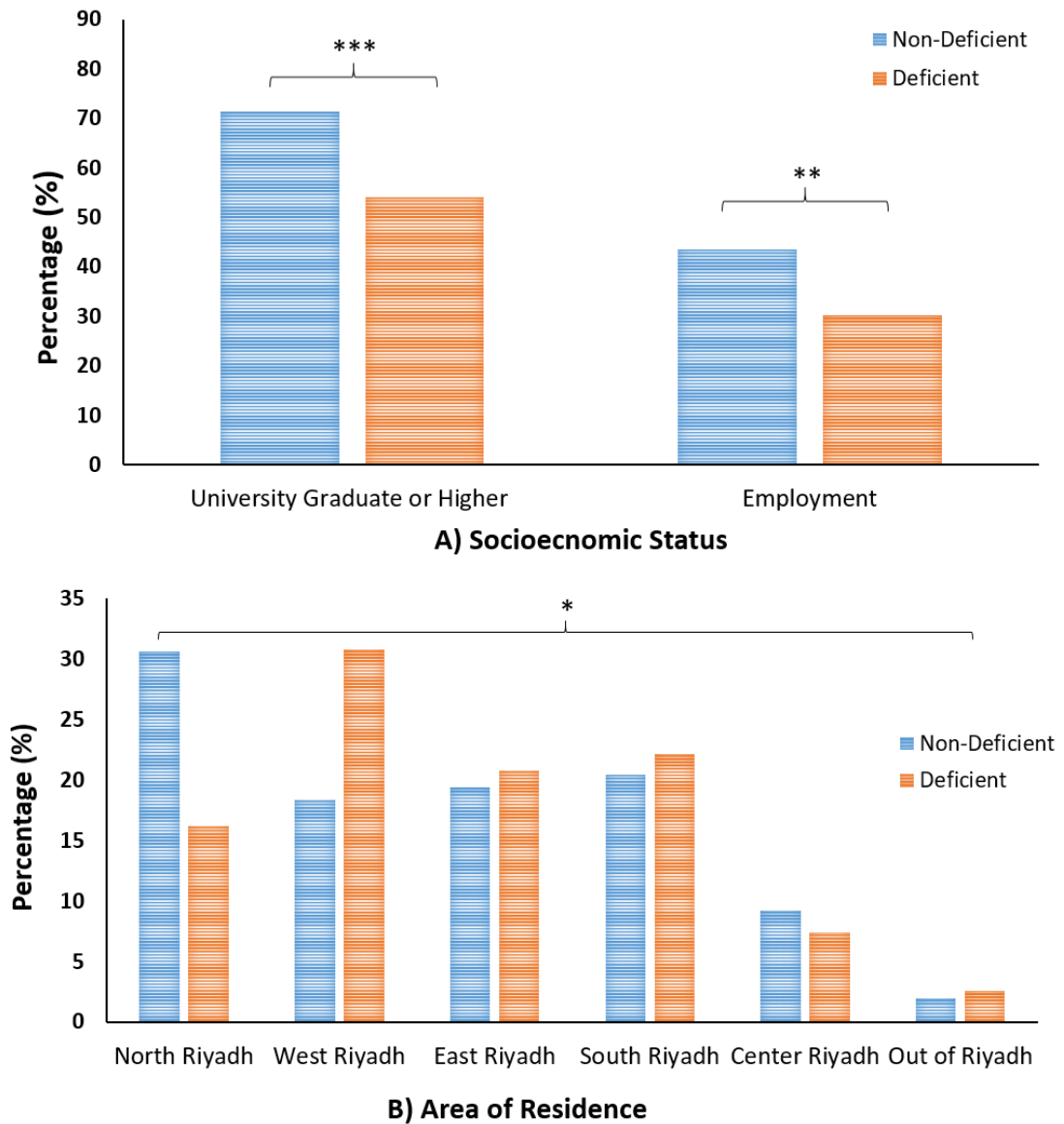
**Figure 3.10** Vitamin D status in early pregnancy

### ***3.3.2.1 Socio-economic Status in Relation to Vitamin D Status***

Proportionally more women were non-deficient in vitamin D if educated to graduate or postgraduate level, compared to the deficient group (71.3% vs. 54%,  $p < 0.001$ ). Similarly, the pregnant women who were non-deficient in vitamin D were more likely to be employed than those in the deficient group (43.5% vs. 30.2%,  $p = 0.008$ ). Moreover, a significantly higher percentage of pregnant women who were non-deficient in vitamin D lived in North Riyadh (30.6%), compared to 16.2% in the deficient group ( $p = 0.001$ ), while around 18.4% of the pregnant women who were non-deficient in vitamin D lived in West Riyadh, compared to 30.8% from the deficient group ( $p = 0.014$ ). However, no differences were observed in income between the groups. Furthermore, the pregnant women living in North Riyadh were significantly more educated, at least to graduate level ( $p = 0.001$ ), in comparison with their counterparts living in West Riyadh ( $p = 0.002$ ) (**Figure 3.11** compares data on education, and area of residence for the deficient and non-deficient groups of pregnant women).

### ***3.3.2.2 Obstetric and Family History in Relation to Vitamin D Status***

Obstetric parameters, such as parity, incidence of caesarean section, and miscarriage, as well as family history of diabetes and obesity, failed to reveal any differences between the groups. However, the rate of nulliparous pregnancy was higher in the non-deficient group compared with the deficient group, although this was not statistically significant (45.9% vs. 35.3%,  $p = 0.127$ ).



**Figure 3.11** (A) Educational status and employment, and (B) Area of residence according to vitamin D status in early pregnancy. The values show the percentage of university graduates and postgraduates, employed women, and women living in different areas of Riyadh, amongst the vitamin D-deficient and non-deficient subjects. P-values: \* denotes  $p < 0.05$ , \*\* denotes  $p < 0.01$  and \*\*\* denotes  $p < 0.001$ , using a Pearson's chi-square test.

### ***3.3.2.3 Anthropometric and Biochemical Characteristics Against Vitamin D Status***

The anthropometric and biochemical characteristics of the deficient and non-deficient groups of pregnant women investigated here are shown in **Table 3.2**. Age (in years) did not show a significant difference between the deficient and non-deficient groups ( $p = 0.192$ ). However, gestational age (in weeks) was significantly higher in the deficient group, compared to the non-deficient group, even after adjustment for BMI and age ( $p = 0.043$ ). Pre-pregnancy BMI, BMI in early pregnancy, WHR, and blood pressure also failed to reveal any differences between the two groups, although body fat percentage was significantly higher in the non-deficient group, even after adjusting for age and BMI ( $35.4 \pm 5.1$  vs.  $34.1 \pm 5.6$ ,  $p = 0.042$ ). In addition, maternal vitamin D levels showed a significant positive correlation with body fat percentage ( $r = 0.13$ ,  $p = 0.003$ ).

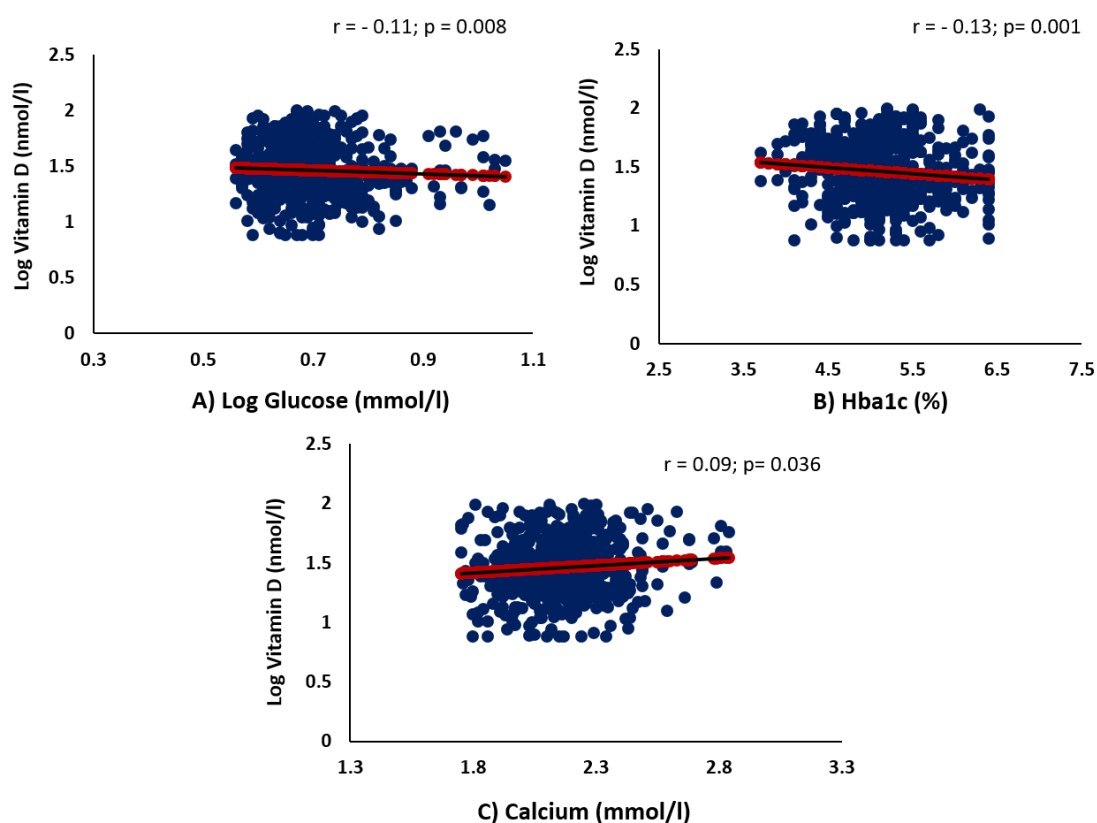
The biochemical profile of the pregnant women showed a vitamin D level of 64.5 nmol/L (57.0-76.6) in the non-deficient group, compared with 24.4 nmol/L (18.0 – 33.76) in the deficient group. HDL-cholesterol was significantly lower in the deficient group ( $p = 0.026$ ), after adjusting for age and BMI. Furthermore, the total cholesterol/HDL ratio was significantly higher in the deficient group ( $p < 0.001$ ). Maternal vitamin D levels, therefore, showed a significant positive correlation with HDL-cholesterol ( $r = 0.19$ ,  $p < 0.001$ ), whereas a significant negative correlation was observed in the vitamin D and total cholesterol/HDL ratio, random glucose, and HbA1c ( $r = -0.15$ ,  $p < 0.001$ ;  $r = -0.11$ ,  $p = 0.008$ ;  $r = -0.13$ ,  $p = 0.001$ ) (**Figure 3.12**).

**Table 3.2 Anthropometrics and biochemical characteristics per vitamin D status in early pregnancy**

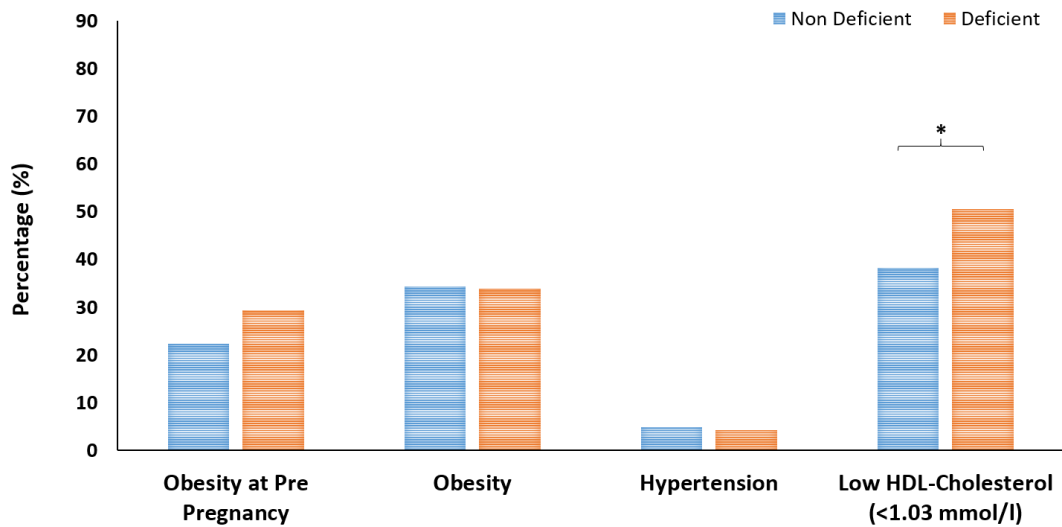
	Non-Deficient (N=110)	Deficient (N = 468)	P-value	P-value*
<b>Anthropometric Parameters</b>				
Age (Years)	29.4 ± 5.2	28.6 ± 5.5	0.192	---
<b>Gestational age (weeks)</b>	11.5 ± 2.9	12.1 ± 3.0	0.066	<b>0.043</b>
Pre-pregnancy BMI (kg/m <sup>2</sup> )	27.1 ± 6.2	27.0 ± 5.9	0.944	0.065
BMI at 1st visit (kg/m <sup>2</sup> )	28.3 ± 6.3	28.0 ± 6.3	0.575	---
Waist circumference (cm)	93.1 ± 12.4	91.1 ± 13.8	0.178	0.102
Hips circumference (cm)	108.8 ± 11.6	107.7 ± 12.1	0.371	0.389
Waist-hip Ratio	0.9 ± 0.1	0.8 ± 0.1	0.336	0.247
Systolic BP (mm Hg)	114.3 ± 13.3	113.8 ± 12.6	0.703	0.801
Diastolic BP (mm Hg)	67.1 ± 9.8	68.0 ± 9.6	0.376	0.248
<b>Body fat (%)</b>	35.4 ± 5.1	34.1 ± 5.6	0.03	<b>0.042</b>
<b>Biochemical Parameters</b>				
<b>Vitamin D (nmol/l) #</b>	64.5 (57.0 – 76.6)	24.4 (18.0 – 33.76)	< 0.001	<b>&lt;0.001</b>
Corrected calcium (mmol/l)	2.3 ± 0.2	2.2 ± 0.2	0.442	0.508
Phosphorus (mmol/l)	1.2 ± 0.5	1.1 ± 0.4	0.127	0.053
Alkaline Phosphatase (mmol/l)	9.3 ± 2.9	9.7 ± 3.4	0.266	0.168
Creatinine (μmol/l)	55.4 ± 17.9	55.9 ± 18.3	0.806	0.914
Glucose (mmol/l) #	4.7 (4.4 - 5.2)	4.8 (4.4 - 5.3)	0.627	0.708
Insulin (uU/mL) #	7.9 (4.6 - 17.7)	8.7 (4.9 - 18.4)	0.427	0.446
HbA1c (%)	5.1 ± 0.5	5.1 ± 0.5	0.490	0.380
Total cholesterol (mmol/l)	5.2 ± 0.8	5.2 ± 1.0	0.748	0.911
<b>HDL-cholesterol (mmol/l)</b>	1.4 ± 0.3	1.3 ± 0.4	0.035	<b>0.026</b>
<b>Cholesterol-HDL Ratio</b>	3.8 ± 0.7	4.1 ± 1.1	< 0.001	<b>0.001</b>
LDL-cholesterol (mmol/l)	3.1 ± 0.6	3.2 ± 0.8	0.681	0.622
Triglycerides (mmol/l)	1.4 ± 0.6	1.4 ± 0.6	0.941	0.728

**Note:** Data presented as a mean and standard deviation for normal variables, while median, first and third quartiles are presented for non-normally distributed variables. # indicates non-normally distributed variables; the p-value for mean differences was obtained from an independent sample t-test for normal variables, and a Mann-Whitney U test for non-normally distributed variables, while \* indicates p-values adjusted for age and BMI on the 1<sup>st</sup> visit through an ANCOVA.

Calcium and phosphorus appeared higher in the non-deficient compared with the deficient group after adjustments, although this difference was not significant ( $p = 0.054$  and  $p = 0.053$ , respectively). Alkaline phosphatase levels were higher in the deficient than in the non-deficient group, but again was not significant ( $p = 0.168$ ). Maternal vitamin D levels showed a significant positive correlation with calcium, phosphorus, and creatinine ( $r = 0.09$ ,  $p = 0.036$ ;  $r = 0.10$ ,  $p = 0.026$ ;  $r = 0.10$ ,  $p = 0.025$ ), respectively. The presence of metabolic disorders according to vitamin D status is displayed in **Figure 3.13**.



**Figure 3.12** Baseline correlations between Log vitamin D (nmol/L) versus (A) glucose, (B) HbA1c, and (C) calcium in early pregnancy.



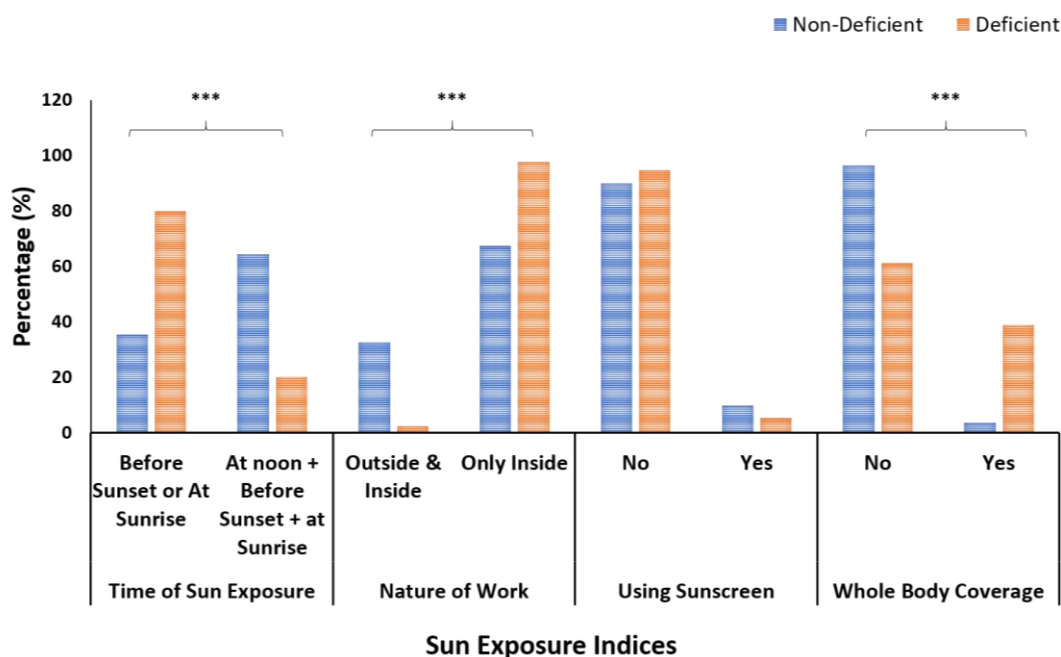
**Figure 3.13** Presence of metabolic disorders according to vitamin D status. P-values: \* denotes  $p < 0.05$ . The values show the percentage of women with different metabolic disorders in early pregnancy, in both vitamin D deficient women and women without deficiency. P-values: \* denotes  $p < 0.05$  according to Pearson's chi-square test.

#### 3.3.2.4 Lifestyle Factors in Relation to Vitamin D Status

Exposure to the sun at noon alone, and exposure at noon and sunset or sunrise, was significantly higher amongst the non-deficient subjects, in comparison with their deficient counterparts (64.5% vs. 20.1%,  $p < 0.001$ ). Similarly, the number of women undertaking most of their work indoors was higher amongst the deficient participants, compared to the non-deficient subjects (97.6% vs. 67.3%,  $p < 0.001$ ). Moreover, the number of subjects covering their whole body with clothing was significantly higher in the deficient group, compared to the non-deficient participants (38.9% vs. 3.6%,  $p < 0.001$ ). The percentage of participants who exposed themselves to the sun (32.7%) was higher in the non-deficient group, compared to the deficient group (25.2%), although this difference was not statistically significant ( $p = 0.109$ ). In addition, 10% of the non-deficient group used sunscreen, compared to 5.3% in the deficient group, but this was not statistically significant ( $p = 0.069$ ). The season in which the sun exposure took place (summer/winter) did not demonstrate any differences between the groups. The samples



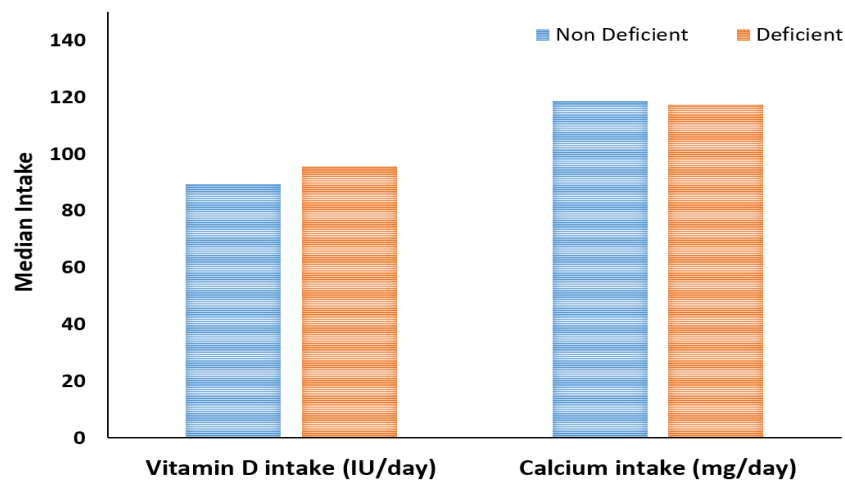
collected in summer amounted to 38.9% in the deficient group, compared to 41.5% in the non-deficient group, whereas in winter, 61.6% of the samples were collected for the deficient participants, compared to 58.5% for the non-deficient group ( $p = 0.623$ ).



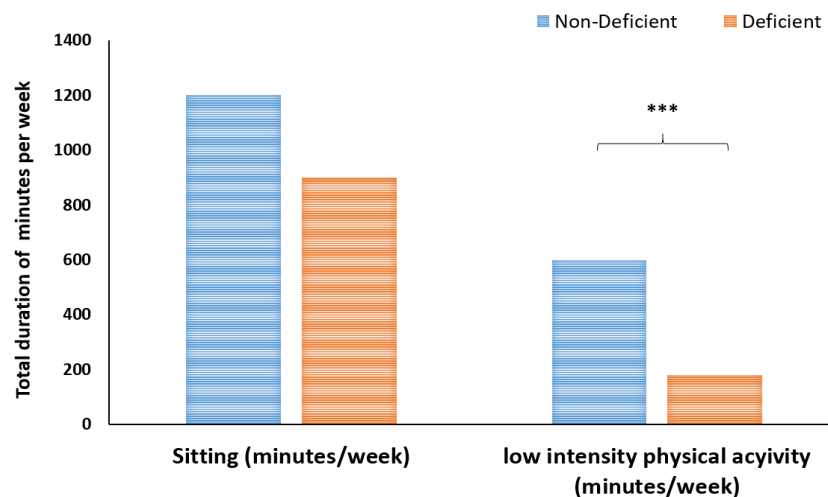
**Figure 3.14** Sun exposure during early pregnancy in relation to vitamin D status during this period. The values show the percentage of women with different sun exposure indices, in both vitamin D-deficient and non-deficient women. Noon time indicates the time between 10 am -2 pm. P-values: \*\*\* refers to  $p < 0.001$  from Pearson's chi-square test.

The current study observed that the vitamin D and calcium intake, estimated in early pregnancy, did not reveal any statistically significant differences between the deficient and non-deficient groups (**Figure 3.15**). This was also true for multivitamin intake reported in early pregnancy (non-deficient at 28.6% vs. deficient at 24.1%). Interestingly, the median number of minutes for women engaged in low-intensity physical activity/walking (minutes/week) was significantly higher in the non-deficient than in the deficient group, even after adjusting for age and BMI (600 vs. 180 minutes/week,  $p < 0.001$ ). In fact, maternal vitamin D levels indicated a significant positive correlation with low intensity physical activity (minutes/week:  $r = 0.32$ ,  $p < 0.001$ ).

0.001). However, the median sedentary time spent (minutes/week) showed no differences between the deficient and non-deficient groups (1200 vs. 900 minutes/week,  $p = 0.669$ ) (**Figure 3.15**).



**Figure 3.15** Estimated vitamin D and calcium intake, according to vitamin D status in early pregnancy. The values show the median intake of vitamin D and calcium from food, in both vitamin D-deficient and non-deficient women. The test used was the Mann-Whitney U test.



**Figure 3.16** Physical activity in relation to vitamin D status during early pregnancy. The values show median sedentary and physical activity in minutes per week for both the vitamin D-deficient and non-deficient groups using the Mann-Whitney U test. P-values: \*\*\* denotes  $p < 0.001$  after adjusting for age and BMI, with ANCOVA.

### 3.3.3 Predictors of Vitamin D Deficiency in Early Pregnancy

Significant independent variables for vitamin D deficiency were assessed using multivariate logistic regression analysis, and adjusted for confounding factors. Significant independent variables that increased the risk of vitamin D deficiency included working exclusively indoors (OR 25.37, 95% CI 5.49-117.28,  $p < 0.001$ ), whole body coverage with clothing (OR 17.81, 95% CI 2.29-138.50,  $p < 0.001$ ), multiparity (OR 3.97, 95% CI 1.66-9.48,  $p = 0.002$ ), total cholesterol/HDL ratio ( $>3.5$ ) (OR 3.30, 95% CI 1.38-7.90,  $p = 0.007$ ), low HDL-cholesterol (OR 2.81, 95% CI 1.22-6.42,  $p = 0.015$ ), and living in West Riyadh (OR 2.00, 95% CI 1.14-3.53,  $p = 0.011$ ) (**Table 3.3**).

The variables that appeared to confer some protection against vitamin D deficiency in early pregnancy were low-intensity physical activity/walking ( $\geq 210$  minutes/week) (OR 0.20, 95% CI 0.09-0.47,  $p < 0.001$ ), sun exposure at noon alone, or with additional exposure at sunrise or sunset (OR 0.24, 95% CI 0.10-0.57,  $p = 0.001$ ), being a university graduate or higher (OR 0.32, 95% CI 0.12-0.86,  $p = 0.023$ ), residence in North Riyadh (OR 0.35, 95% CI 0.15-0.79,  $p = 0.012$ ), and age (OR 0.91, 95% CI 0.83-1.00,  $p = 0.051$ ) (**Table 3.3**).

**Table 3.3 Predictors of vitamin D deficiency among pregnant women in early pregnancy**

Parameters	Unadjusted OR (95% CI)	P-value	Model 1 OR (95% CI) *	P-value*	Model 2 OR (95% CI) **	P-value**
Age	0.97 (0.94 – 1.01)	0.192	0.98 (0.94 – 1.02)	0.311	0.91 (0.83 – 1.00)	0.051
Gestational age	1.07 (0.99 – 1.15)	0.066	1.07 (1.00 – 1.15)	0.059	0.99 (0.86 – 1.13)	0.881
<b>Multiparity</b>	1.56 (1.00 – 2.43)	0.051	2.02 (1.21 – 3.36)	0.007	3.97 (1.66 – 9.48)	<b>0.002</b>
Pre-pregnancy BMI (kg/m <sup>2</sup> )	1.00 (0.96 – 1.03)	0.944	1.00 (0.97 – 1.04)	0.867	0.99 (0.92 – 1.06)	0.729
Obesity by 1 <sup>st</sup> visit	0.86 (0.55 - 1.35)	0.517	1.22 (0.57 – 2.61)	0.604	0.95 (0.41 – 2.19)	0.907
<b>University graduate or postgraduate</b>	0.47 (0.30 - 0.75)	0.001	0.42 (0.26 - 0.68)	<0.001	0.32 (0.12 - 0.86)	<b>0.023</b>
Employment	0.56 (0.36 - 86)	0.008	0.56 (0.36 - 0.88)	0.011	0.61 (0.27 – 1.34)	0.605
<b>Living in North Riyadh</b>	0.44 (0.27 - 0.73)	0.001	0.46 (0.27 - 0.77)	0.003	0.35 (0.15 - 0.79)	<b>0.012</b>
<b>Living in West Riyadh</b>	1.41 (1.07 - 1.85)	0.014	1.38 (1.05 - 1.83)	0.024	2.00 (1.14 - 3.53)	<b>0.016</b>
HbA1c at 1 <sup>st</sup> visit	1.14 (0.78 - 1.68)	0.490	1.25 (0.83 - 1.88)	0.278	1.34 (0.62 - 2.90)	0.462
Random glucose (mmol/l) at 1 <sup>st</sup> visit	1.02 (0.85 - 1.23)	0.627	1.03 (0.85 - 1.26)	0.750	0.84 (0.61 - 1.17)	0.297
TG (≥ 1.7 mmol/l)	1.06 (0.67 – 1.69)	0.800	1.11 (0.68 – 1.80)	0.687	0.62 (0.24 – 1.59)	0.618
<b>Total cholesterol/HDL ratio (&gt;3.5)</b>	1.37 (0.89 – 2.11)	0.158	1.50 (0.96 – 2.35)	0.076	3.30 (1.38 – 7.90)	<b>0.007</b>
Low HDL-cholesterol (< 1.03 mmol/l)	1.66 (1.09 - 2.54)	0.019	1.74 (1.12 - 2.69)	0.014	2.81 (1.22 – 6.42)	0.015
Hypertension (HTN)	0.86 (0.31 – 2.36)	0.770	0.90 (0.32 – 2.55)	0.843	5.07 (0.23 – 110.38)	0.302
Blood sample collection (Summer)	1.11 (0.73 - 1.71)	0.623	1.05 (0.67 - 1.64)	0.847	1.18 (0.50 - 2.74)	0.698
Sun exposure	0.69 (0.44 - 1.09)	0.109	0.67 (0.42 - 1.06)	0.083	1.20 (0.50 - 2.89)	0.688
<b>Time of exposure (at noon)</b>	0.14 (0.09 - 0.22)	<0.001	0.14 (0.09 - 0.23)	<0.001	0.24 (0.10 - 0.57)	<b>0.001</b>
<b>Nature of work (indoors)</b>	20.21 (9.85 - 41.46)	<0.001	20.62 (9.72 - 43.78)	<0.001	25.37 (5.49 – 117.28)	<b>&lt;0.001</b>
<b>Coverage with clothing (whole body)</b>	16.86 (6.11 - 46.55)	<0.001	16.47 (5.92 – 45.80)	<0.001	17.81 (2.29 – 138.50)	<b>0.006</b>
Vitamin D intake (> 600 IU/day)	0.35 (0.11 - 1.12)	0.064	0.42 (0.13 - 1.40)	0.158	0.35 (0.07 - 1.93)	0.230
Calcium Intake (> 1000 mg/day)	0.62 (0.23 - 1.64)	0.329	0.74 (0.27 - 2.01)	0.560	0.86 (0.14 - 5.14)	0.870
Use of multivitamin supplements	0.80 (0.46 - 1.39)	0.421	0.78 (0.43 - 1.40)	0.400	0.62 (0.25 - 1.54)	0.299
<b>Low intensity physical activity (≥210 minutes/week)</b>	0.28 (0.17 - 0.47)	<0.001	0.31 (0.18 – 0.51)	<0.001	0.20 (0.09 - 0.47)	<b>&lt;0.001</b>

**Note:** Odds ratio (OR) and 95% CI for OR were obtained using logistic regression analysis, taking vitamin D deficiency (< 50nmol/L) as a dependent variable against potential risk factors, and as an independent risk. Model 1 is for age, BMI, and sun exposure. Model 2 is Model 1+ parity, summer season, vitamin D intake, multivitamin intake, physical activity, education, employment, living in North, and coverage with clothing. Categorical variables (e.g. education, employment, HTN, sun exposure, and multivitamin supplementation) are presented as 'yes/no'; other categorical values include area of residence in Riyadh (North or West Riyadh vs. all other areas); season blood sample collection (summer vs. winter); time of sun exposure (noon alone plus either sunset or sunrise, or both vs. sunrise or sunset or both); working indoors vs. working indoors + outdoors, and whole body vs. partial coverage with clothing.

### 3.4 Discussion

This is the largest study ever conducted in Saudi Arabia to assess hypovitaminosis D in the early stages of pregnancy, as well as its predictors. It was observed that 81% of the pregnant women studied were deficient in 25(OH)D. Younger women, multiparous subjects, women with a lower level of education, housewives (as opposed to employees outside the home), and living in West Riyadh were all factors noted to be linked with lower vitamin D levels in early pregnancy. In addition, women who were less frequently exposed to the sun due to coverage of their whole body with clothing, and those who spent most of their working lives indoors were more likely to suffer from hypovitaminosis D in early pregnancy. However, a higher amount of physical activity, life in North Riyadh, and sun exposure at noon were associated with higher 25(OH)D concentrations.

Vitamin D deficiency or insufficiency would not usually be expected in a sunny country like Saudi Arabia (latitude 25°N) (Sedrani et al., 1983), however, numerous studies have reported a high prevalence of hypovitaminosis D in the Saudi population, across different ages (Al-Musharaf et al., 2012; Alsuwaida et al., 2013; Al-Zoughool et al., 2015). Nevertheless, only a limited number of studies, using relatively small sample sizes, have studied hypovitaminosis D in pregnant Saudi women to date (Serenius et al., 1984; Taha et al., 1984; Azhar, 2009; Al-Ajlan et al., 2015; Al-Faris, 2016; Al-Shaikh et al., 2016). Moreover, most of these studies measured vitamin D levels during the third trimester, or at the time of delivery, while only two studies assessed 25(OH)D status during early gestation (Al-Ajlan et al., 2015; Al-Faris, 2016). For example, Al-Ajlan et al. (2015) studied 515 pregnant women in their first trimester, and found that 26.2 % were deficient ( $< 25$  nmol/L), 68.0 % were insufficient (25–50 nmol/L), and only 3.5 % had sufficient (50–75 nmol/L) levels of vitamin D. Al-Faris (2016) investigated vitamin D status in 160 pregnant women from Riyadh, and reported that 50% women were vitamin D-deficient ( $< 50$  nmol/L) and 43.8% had insufficient levels (50–74 nmol/L) of vitamin D.

Apart from Saudi Arabia, however, vitamin D deficiency in early pregnancy has also been reported in the UAE (78%), Oman (98%), India (62%), Korea (91.8%), Spain (18%), the UK (57%), and Canada (39%), indicating that inadequate vitamin D levels during gestation is a global concern (Narchi et al., 2010; Al Kalbani et al., 2011; Makgoba et al., 2011; Dasgupta et al., 2012; Wei et al., 2012; Choi et al., 2015; Rodriguez et al., 2016). The rates detected in the existing study are comparable to those revealed for other Gulf and Asian countries.

A large variety of risk factors for suboptimal vitamin D levels, such as latitude, season, clothing, sunscreen use, skin pigmentation, atmospheric components, and air pollution are directly or indirectly linked to sunlight exposure (Holick, 2007; Christakos et al., 2010; Wacker & Holick, 2013). In the present study, the season (winter/summer) did not imply any significant differences in vitamin D levels, between the deficient and non-deficient pregnant women. However, this was anticipated, because although the season would indicate the amount of available sunlight, coverage of the whole body with clothing, and a large proportion of domestic or other work being performed indoors in Saudi Arabia, mean that exposure indices are largely unaffected by season amongst the Saudi population. Moreover, Saudis generally avoid the hot sun in summer. Therefore, inadequate exposure to sunlight, measured using several different indices, was shown to be a major risk factor for suboptimal vitamin D levels in the current study. In addition, the timing of the sun exposure in this study (noon) was also critical for determining the vitamin D levels, and this corresponded with previous data, where subjects were found to spend time outside in summer during the peak vitamin D production time of 10 am – midday. In contrast, this production time peaks at around 11 am in winter (Alshahrani et al., 2013). Therefore, despite abundant sunlight in Saudi Arabia, lifestyle patterns and traditional dress, covering the whole body, significantly decreases sun exposure. This was evident in the present study, where the pregnant subjects, who covered their whole body, had significantly lower levels of vitamin D.

Saudi women use dark veils to cover their whole body because of cultural and religious beliefs. Limiting sun exposure through the use of these veils has been implicated as a major risk factor for vitamin D deficiency (Azhar, 2009; Al-Ghamdi et al., 2012; Al-

Mogbel, 2012). In fact, Muslim women all over the world, who resort to full body coverage, due to their religious beliefs, are consequently at risk of developing vitamin D deficiency (Ates et al., 2016; Karras et al., 2016). In Saudi Arabia and elsewhere, people have also adopted sun avoidance behaviour, due to modern lifestyles based on indoor work, such as in office work. This is especially true in Saudi Arabia, which has a very hot climate for a large part of the year. The present study, supported by others, indicates that pregnant women working indoors are at greater risk of having low vitamin D levels (Xiang et al., 2013).

This study indicates that vitamin D levels vary in pregnant women, according to their educational status, with a lower level of education increasing the risk of vitamin D deficiency. This observation has been reported in a number of other studies documenting higher vitamin D levels among more educated pregnant women (Johnson et al., 2011; Burris et al., 2012a; Vandevijvere et al., 2012; van den Berg et al., 2013; Fenina et al., 2016; Sharma et al., 2016). Compared to other areas, pregnant women residing in North Riyadh were found to have a higher vitamin D status. This is most likely because North Riyadh is known for being a more affluent part of the city, with more highly educated inhabitants. However, as there were no differences in income observed between the 25(OH)D-deficient and sufficient groups, this was attributed more to the subjects' educational level. In contrast to this, low socio-economic status has been connected to vitamin D deficiency (Aly et al., 2013). Furthermore, in support of this current study, previous research has reported that women who have had two or more previous births are more likely to have lower 25(OH)D<sub>3</sub> concentrations, compared to women who have had just one previous birth (Aly et al., 2013; Andersen et al., 2013).

Dietary intake of vitamin D (2.6%) and calcium (4.6%) intake were shown to be low in this study, as with other studies (Jensen et al., 2012; Al-Faris, 2016; Ayadi et al., 2016). Approximately one quarter (25%) of the pregnant women in the existing study were taking multivitamins in their first trimester, which is similar to other studies from different countries, whereby a range of 7.5%-63.1% of the subjects have been found to use multivitamin supplementation (Vandevijvere et al., 2012; Charatcharoenwitthaya et al., 2013; Al-Faris, 2016; Ates et al., 2016). Collectively, these data suggest that

vitamin D intake during pregnancy, whether from food or supplements, is inadequate across the world (Scholl & Chen, 2009; McGowan et al., 2011; Burris et al., 2012b; Vercruyssen et al., 2012; Karras et al., 2014). This is because dietary sources of vitamin D tend to be limited and comprise less than 10% of the daily vitamin D requirement as well as current food fortification is inadequate in Saudi Arabia (Holick et al., 2012; Kanan et al., 2013). In addition, Riyadh is a landlocked city, with no access to the sea or rivers, so consumption of vitamin D rich fish is usually infrequent (Al-Mogbel, 2012). Moreover, Saudi society has experienced a dramatic change in its dietary and lifestyle patterns, with a shift towards a more unhealthy dietary habits (Al-Turki, 2008; Al-Ghamdi et al., 2012; Al-Faris et al., 2015).

The relationship between 25(OH)D and obesity in pregnancy is inconsistent. This present study failed to find any evidence of an association between vitamin D levels and pre-pregnancy or early pregnancy obesity. These observations are consistent with findings from similar studies (Choi et al., 2015; Flood-Nichols et al., 2015; Al-Faris, 2016; Ates et al., 2016; Rodriguez et al., 2016). Moreover, the mean BMI observed in early pregnancy ( $27 \text{ kg/m}^2$ ) was comparable to another study conducted by Ates et al. (2016) ( $25 \text{ kg/m}^2$ ), where the mean BMI did not reach obesity level. This could explain the absence of any differences in vitamin D deficiency/sufficiency in this regard. However, there is still some evidence suggesting that obesity may be associated with low vitamin D levels (Vandevijvere et al., 2012; Andersen et al., 2013; Karras et al., 2016). Furthermore, compared to the deficient group, the non-deficient group of pregnant women had a higher fat percentage in the present study, even after adjusting for age and BMI. Nevertheless, this was a marginal difference and requires further confirmation through other studies. Fat status during pregnancy may be misleading and difficult to interpret because of the continuous changes in fat status that occurs throughout pregnancy. This is supported by the more marked trend towards pre-pregnancy obesity in the vitamin D-deficient group in this study; a trend that disappeared in early pregnancy, perhaps due to normal physiological changes.

Pregnant women engaging in low-intensity physical activity were also noted to have higher vitamin D levels, compared to those in the deficient group. Physical activity



amounting to at least 30 minutes per day in early pregnancy was determined as independently protecting against vitamin D deficiency. This may be attributed to greater mobilisation of vitamin D from burned fat deposits through increased physical activity (Riedt et al., 2005; Tzotzas et al., 2010). Pregnant women involved in daily outdoor activity during the early stages of pregnancy were more likely to have adequate vitamin D levels (Al-Faris, 2016). Jensen and colleagues (1980) have also proposed that pregnant women performing an outdoor physical activity are more likely to have a satisfactory 25(OH)D status. The elevated vitamin D levels among pregnant women involved in outdoor activity could also be due to greater sunlight exposure, thus triggering vitamin D synthesis (Kluczynski et al., 2011), and as indicated above, it is highly likely that the exercise itself may contribute to the maintenance of vitamin D status (Scragg et al., 1995).

To expand on the above, other related explanations include effects independent of sun exposure possibly impacting on vitamin D levels in relation to physical activity (AlMulhim et al., 2015; Rodriguez et al., 2016). This current study is the first of its kind to assess the impact of adjusted sun exposure and physical activity on vitamin D levels among pregnant women. However, other studies investigating the association between physical activity and vitamin D levels, even after adjusting for sun exposure in non-pregnant females, have revealed similar findings (Scragg & Camargo, 2008; Brock et al., 2010; Kluczynski et al., 2011; Touvier et al., 2015; Wanner et al., 2015). It is possible that a reduction in ionised calcium during exercise stimulates parathyroid hormone release, thus activating  $1,25(\text{OH})_2\text{D}_3$  (Maïmoun & Sultan, 2009). In addition, increased lipolysis following exercise-induced weight loss may also mobilise vitamin D from adipose tissues, resulting in elevated serum levels of vitamin D (Riedt et al., 2005; Zittermann et al., 2009; Tzotzas et al., 2010).

Among the lipid profile parameters, HDL-cholesterol was significantly lower in the deficient group, resulting in an increased total cholesterol/HDL ratio, compared to their non-deficient counterparts. Moreover, low HDL-cholesterol levels were 2.81 times more likely to be associated with vitamin D deficiency, while the total cholesterol/HDL ratio indicated a 3.30 times higher risk of vitamin D deficiency in early pregnancy.

These findings indicate that women with low vitamin D levels are at higher risk of cardiovascular diseases (Boucher, 1998; Barter et al., 2007). Among non-pregnant women, low HDL-cholesterol has been reported as an independent risk factor of vitamin D deficiency (Forrest & Stuhldreher, 2011). Another study looked at 13,039 adults and showed that higher 25(OH)D levels were associated with higher HDL-cholesterol and a lower total cholesterol/HDL ratio, after considering factors such as diabetes, physical activity, BMI, and waist circumference (Faridi et al., 2016). The mechanism of this association is not clear, but vitamin D may indirectly influence lipid metabolism mediated by insulin resistance and inflammation (Guasch et al., 2012). Matsuura and colleagues (2006) proposed that vitamin D may regulate macrophage function of reverse cholesterol transport, and the number of large HDL particles may be elevated, by taking over cholesterol through macrophages. Zhou and colleagues (2008) revealed that vitamin D possibly improves free fatty acid-induced insulin resistance. Meanwhile, Kang et al. (2012) suggest that vitamin D could stimulate cytokine gene expression in macrophage, and perform many systemic anti-inflammatory actions. Most recently, Slominski et al. (2015) reported that novel vitamin D<sub>3</sub>-hydroxyderivatives are non-calcemic, unlike 1,25(OH)<sub>2</sub>D<sub>3</sub>, thus potentially promoting anti-inflammatory activity. To date, however, few studies have explored vitamin D status with lipid profiles during early pregnancy, and longitudinal studies are lacking. Therefore, the present study has narrowed this gap in the literature, by being among the first studies to show that low HDL-cholesterol levels, and total cholesterol/HDL ratios, highly increase the risk of vitamin D deficiency in early pregnancy.

Finally, vitamin D exhibited a negative correlation with random blood glucose and HbA1c in this study, suggesting that the two parameters may be linked with serum vitamin D levels, although this was not statistically significant in multivariate analysis. A negative correlation between vitamin D and HbA1c during the first trimester of pregnancy has been reported previously (Jafarzadeh et al., 2015).

In conclusion, the Saudi pregnant women studied in this instance had a high prevalence of vitamin D deficiency (81%) in their first trimester. Whole body coverage with

clothing, lower levels of physical activity, living in West Riyadh, and multiparity were found to be the key factors predisposing pregnant women to vitamin D deficiency. Vitamin D deficiency was also noted to be associated with adverse HDL-cholesterol status, although this finding requires replication. There appears to be a need to increase awareness among health professionals and the public in general for avoiding vitamin D deficiency, specifically among women and those of childbearing age. Thus, to improve vitamin D status, lifestyle recommendations, such as a combination of increased physical activity, exposure to the sun at noon, consumption of foods rich in vitamin D, and vitamin D supplementation, may all help to raise vitamin D levels. Specific recommendations and policies should be implemented in the KSA to detect and prevent vitamin D deficiency in general, but particularly during pregnancy, in order to avoid the associated adverse maternal and neonatal complications.

## **Chapter 4**

### **Longitudinal Changes in Vitamin D Status During Pregnancy: Comparison Between Early and Mid- Term Pregnancy**

## 4.1 Introduction

Pregnancy itself is particularly associated with substantial changes in vitamin D and calcium metabolism, which are key to foetal bone development. The need to prioritise foetal needs requires the mother to increase her vitamin D intake during pregnancy, as well as during lactation (Brannon & Picciano, 2011; Ross et al., 2011). Understandably, the foetus is entirely dependent on the mother for its supply of both calcium and vitamin D during pregnancy (Lapillonne, 2010). However, despite vitamin D levels having already been determined for nutritional requirements during gestation, these levels seem debatable. In particular, vitamin D deficiency cut-off points for pregnant women have been adapted from studies performed on non-pregnant populations (Dror & Allen, 2010; Flood-Nichols et al., 2015). At present, in the non-pregnant population, a circulating level of at least 50 nmol/L is considered adequate to avoid bone dysfunction, although ideally 75 nmol/L of vitamin D is considered sufficient (Bodnar & Simhan, 2010; Holick et al., 2011; Ross et al., 2011).

Despite knowledge of the vitamin D levels possibly required in pregnancy, the prevalence of vitamin D deficiency remains high, during early pregnancy (Dasgupta et al., 2012), mid-term pregnancy (Xiao et al., 2015), and late pregnancy (Al-Shaikh et al., 2016), although a limited amount of research has addressed the determinants of 25(OH)D deficiency during mid-pregnancy. Studies assessing 25(OH)D levels among pregnant women in mid-late pregnancy have reported a 48-80% prevalence of vitamin D deficiency (Sahu et al., 2009; Perez-Ferre et al., 2012; Bener et al., 2013; Gernand et al., 2013; Choi et al., 2015; Xiao et al., 2015). In one Middle Eastern study from Qatar, which assessed mid-pregnancy, this deficiency has been attributed to inadequate sun exposure, physical inactivity, and a lack of dietary vitamin D (Bener et al., 2013). Additionally, low socio-economic status has been linked with vitamin D deficiency during early-mid pregnancy in one study (Shand et al., 2010).

However, it should be stressed here that not all studies have provided consistent evidence on the changing level of 25(OH)D as pregnancy progresses. Some studies suggest that 25(OH)D levels decline during pregnancy (Ardawi et al., 1997; Zhang et

al., 2014), while other data indicate that vitamin D levels are elevated during pregnancy (Sanchez et al., 1997; Bärebring et al., 2016), and some have revealed no change (Reddy et al., 1983; Seely et al., 1997; Marwaha et al., 2011). These apparent conflicts may be due to various influences on the populations studied.

To elaborate on the above, vitamin D levels during pregnancy can be influenced by the seasonal and lifestyle factors prevailing at the time. Factors which can adversely disturb 25(OH)D status during pregnancy, include ethnicity, less than optimal sun exposure, clothing style, and low dietary or supplemented vitamin D intake (Bärebring et al., 2016). On the other hand, pregnancy-related factors influencing vitamin D status could include haemodilution, hormonal changes, increased maternal and foetal demand, increased body fat, and reduced outdoor activity (Bodnar et al., 2007b; Christesen et al., 2012). Alterations in metabolic profile during pregnancy, due to changes in oestrogen, progesterone, and insulin homoeostasis have also been linked to reduced vitamin D levels (Kalkhoff, 1982). The relationship between adverse metabolic profiles and reduced vitamin D levels have been observed in non-pregnant and pregnant female populations alike (Ford et al., 2005; Maghbooli et al., 2008). Finally, it has been shown that the active trans-placental transport of calcium to the developing foetus may influence vitamin D levels (Jones et al., 2000). Therefore, the overall conclusion is that adequate vitamin D has a favourable influence on the course of a pregnancy, maternal and neonatal calcium homoeostasis, and bone maturation and mineralisation (Jones et al., 2000; Walicka & Marcinowska-Suchowierska, 2008).

Studies measuring vitamin D during pregnancy have been primarily concerned with the association between adverse maternal and foetal outcomes. However, measuring vitamin D just once, and in a single trimester will not necessarily reflect vitamin D levels across an entire pregnancy. Nevertheless, it would appear that vitamin D levels recorded at different time points during the first half of gestation may give a better indication and prediction of 25(OH)D status across the entire term. This is due to a higher demand for nutrients by the foetus, since over 90% of foetal growth takes place at end of pregnancy (Engelking & Rebar, 2012). Furthermore, data on the determinants of 25(OH)D deficiency from the first to the third trimester are limited (Moon et al.,

2015; Bärebring et al., 2016), and no studies have evaluated determinants for vitamin D deficiency from early to mid-gestation. Additionally, there are no available data on vitamin D levels in pregnancy, followed up after controlling for different lifestyle factors. Moreover, in Saudi Arabia to date, no study has measured 25(OH) levels mid-pregnancy, or 25(OH)D status prospectively in a large Saudi cohort of pregnant women. This Chapter presents novel insights in a first study comparing vitamin D levels in early and mid-gestation in Saudi Arabia, as well as the maternal risk factors potentially influencing this relationship.

## **4.2 Research Design and Methods**

This prospective study followed 383 pregnant women from first to the second visit. However, 45 of the subjects were excluded, as they reported taking vitamin D supplementation by mid-pregnancy and 41 were excluded, due to their questionnaires being incomplete. The corresponding analysis was, therefore, conducted on 297 pregnant Saudi women.

### **4.2.1 Data Collection**

Clinical assessments were conducted on two occasions. Baseline, non-fasting, blood samples were obtained during the first visit at 8-12 weeks of gestation. The second visit was scheduled for the second trimester, at 24-28 weeks, and the women were asked to fast for at least 10 hours. Anthropometric and biochemical assessments, as well as questionnaire-based interviews, were conducted at both time points. GWG and changes in body fat were calculated as explained in Chapter 2. As described earlier, the interview questionnaires included a FFQ, questions on sun exposure, and a physical activity questionnaire (IPAQ) (Al-Daghri et al., 2011; Harrison et al., 2011; Al-Musharaf et al., 2012; Al-Othman et al., 2012b; Takahasi et al., 2013).

### **4.2.2 Laboratory Analysis**

During the first visit, random blood samples were collected to measure total 25(OH) D, calcium, phosphorus, albumin, creatinine, and alkaline phosphatase along with glycaemic and lipid profile. For mid-pregnancy measurements, the biochemical parameters assessed during the first visit were repeated. The analysis of previous labs were detailed in Chapter 2. Metabolic syndrome was assessed in the second visit and classified based on criteria suggested by the US NHLBI/AHA (Grundy et al., 2005).

### **4.2.3 Data Analysis**

Data were analysed using SPSS version 21.0 statistical software (SPSS, Chicago, IL, USA). Pearson's chi-square test was conducted to identify any association between categorical variables (deficient and non-deficient). To determine differences between



the groups, an independent sample t-test and Mann-Whitney U Test was used for normal and abnormal variables, respectively. Adjustment for age and BMI between the groups was done using ANCOVA. Comparison of median vitamin D levels from early to mid-pregnancy was done using the Wilcoxon signed rank test, and adjustment for mid-pregnancy gestational age, multivitamin intake, physical activity, estimated vitamin D intake, sun exposure, and season, was done using repeated measures ANCOVA. The difference between two visits was then measured using a paired sample t-test and Wilcoxon signed rank test for normal and abnormal variables, respectively.

Pearson's and Spearman's rank correlation coefficients were also determined to assess the linear relationship between quantitative variables for normally and non-normally distributed variables, respectively. Logistic regression was used to identify the risk factors associated with vitamin D deficiency by mid-pregnancy. This method used the presence or absence of vitamin D deficiency as a dichotomous variable. Possible confounding factors, such as age, BMI, sun exposure, GWG, parity, season, vitamin D intake, multivitamins, physical activity, education, employment, residence in North Riyadh, and coverage with clothing, were included in the analysis. A p-value of  $< 0.05$  and 95% confidence intervals were applied to report the statistical significance, and precision of the estimates.

## 4.3 Results

### 4.3.1 Comparison Between Attendees at the Early and Mid-Pregnancy Visits and Absentees from the Mid-Pregnancy Visit

Of the 578 pregnant women who had previously attended an early pregnancy visit, 383 later attended a mid-pregnancy visit, as described in the CONSORT diagram in Chapter 2 (**Figure 2.1**). The subjects who attended the mid-pregnancy visit displayed a trend towards higher education (59.7% vs. 52.7%,  $p = 0.116$ ), and higher income (13.4% vs. 9.3%,  $p = 0.180$ ), compared to the subjects who failed to attend the visit, although this was not statistically significant. The percentage of subjects living in West Riyadh was significantly higher amongst the women who failed to attend the visit, compared to those who did (33.9% vs. 25.7%,  $p = 0.049$ ). However, no difference was observed in employment between the women attending mid-pregnancy and those failing to attend. Moreover, the subjects who failed to attend the visit had a significantly higher rate of previous miscarriage (38.6% vs. 26.6%,  $p = 0.022$ ), and family history of obesity (25.6% vs. 14.1%,  $p = 0.011$ ), compared to those who attended. A family history of diabetes appeared to be slightly more common amongst the subjects who did not attend, compared to those who did, although this was not statistically significant (78.6% vs. 70.1%,  $p = 0.059$ ).

The mothers who did not attend the mid-pregnancy visit were significantly younger than those who attended ( $28.1 \pm 5.6$  vs.  $29.1 \pm 5.3$  years old,  $p = 0.041$ ), but no differences were observed in the anthropometrics of either group. The expectant mothers who did not attend the mid-pregnancy visit had significantly higher corrected serum calcium ( $2.3 \pm 0.2$  vs.  $2.2 \pm 0.2$  mmol/L,  $p = 0.014$ ), and phosphorus levels ( $1.2 \pm 0.4$  vs.  $1.1 \pm 0.4$  mmol/L,  $p = 0.036$ ), and their median vitamin D concentration (29.7 vs. 27.7 nmol/L) was higher than that of the women who attended, but this was not statistically significant ( $p = 0.171$ ) (**Table 4.1**). The median estimated dietary intake of vitamin D and calcium amongst the subjects, who did not attend, was significantly lower than that of the subjects who completed the study (85.1 vs. 105.4 IU/day,  $p <$

0.001 and 102.9 vs. 128.9 mg/day,  $p < 0.001$ , respectively). However, no difference was detected in their physical activity or sun exposure variables.

**Table 4.1 Maternal characteristics in early pregnancy, relative to attendance or non-attendance of the mid-pregnancy visit**

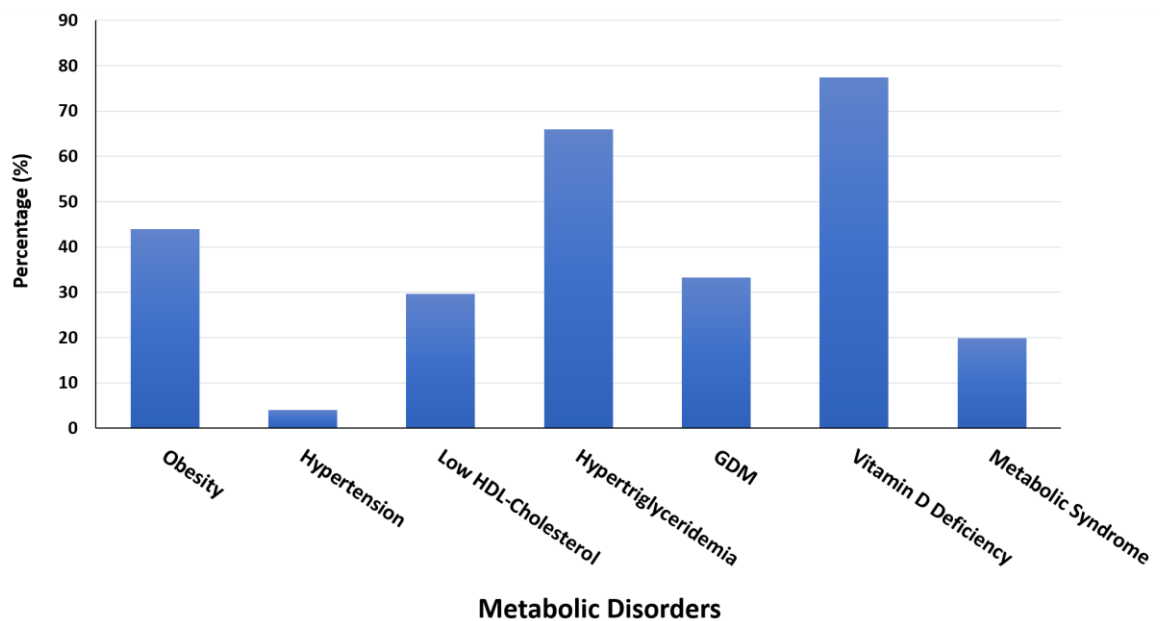
	Attending (N=383, as per initial inclusion)	Non-attending (N=195)	P-value	*P-value
<b>Anthropometric Parameters</b>				
Age (in years)	29.1 ± 5.3	28.1 ± 5.6	0.041	---
Pre-pregnancy BMI (kg/m <sup>2</sup> )	27.0 ± 6.0	27.0 ± 5.9	0.878	0.087
BMI (kg/m <sup>2</sup> )	28.1 ± 6.3	27.8 ± 6.2	0.588	---
Waist-hip ratio (WHR)	0.9 ± 0.1	0.8 ± 0.1	0.190	0.273
Systolic BP	113.8 ± 13.2	114.2 ± 11.6	0.737	0.687
Diastolic BP	67.4 ± 9.5	68.9 ± 9.8	0.080	0.061
Body fat %	34.1 ± 5.6	34.8 ± 5.5	0.186	0.065
<b>Biochemical Parameters</b>				
Glucose (mmol/L) #	4.8 (4.4 - 5.3)	4.8 (4.3 - 5.4)	0.661	0.902
Insulin (uU/mL) #	8.2 (4.6 - 18.3)	8.7 (5.0 - 18.4)	0.861	0.775
HbA1c (%)	5.1 ± 0.5	5.1 ± 0.6	0.925	0.744
Total cholesterol (mmol/L)	5.2 ± 1.0	5.1 ± 1.1	0.399	0.827
HDL-cholesterol (mmol/L)	1.3 ± 0.4	1.3 ± 0.3	0.717	0.855
Cholesterol-HDL ratio	4.0 ± 1.1	4.0 ± 1.0	0.566	0.899
LDL-cholesterol (mmol/L)	3.2 ± 0.7	3.1 ± 0.8	0.357	0.716
Triglycerides (mmol/L)	1.4 ± 0.6	1.4 ± 0.6	0.500	0.946
Vitamin D (nmol/L) #	27.7 (18.8 - 43.6)	29.7 (21.3 - 44.9)	0.171	0.154
<b>Corrected calcium (mmol/L)</b>	2.2 ± 0.2	2.3 ± 0.2	0.002	<b>0.014</b>
<b>Phosphorus (mmol/L)</b>	1.1 ± 0.4	1.2 ± 0.4	0.021	<b>0.036</b>

**Note:** Data are presented as mean and standard deviation for normal variables, while the median, first, and third quartiles are presented for non-normally distributed variables. # indicates non-normally distributed variables; the p-value for mean differences was obtained through an independent sample t-test for normal variables, and a Mann-Whitney U Test for non-normally distributed variables. \* indicates p-values adjusted for age and BMI at the first visit using ANCOVA.

### 4.3.2 General Characteristics of Subjects at Mid-pregnancy

#### 4.3.2.1 Anthropometric and Biochemical Characteristics

In total, 297 pregnant Saudi women successfully attended their second visit. Their mean age was  $28.9 \pm 5.3$  years, and the mean gestational age was  $26.3 \pm 3.2$  weeks. Anthropometric and biochemical parameters during mid-pregnancy are shown in **Table 4.2**. Mean BMI mid-pregnancy was  $30 \pm 6.0$  kg/m<sup>2</sup>, comprising 33.8% (100/297) were overweight and 43.9% (130/297) were obese. Moreover, 66% (196/297) of the subjects displayed hypertriglyceridemia, and 29.6% (88/297) had low HDL-cholesterol. GDM was also diagnosed in 33.3% (99/297) of the subjects, based on the IADPSG (**Figure 4.1**), which will be discussed in more detail in Chapter 5. Finally, about 18% of the subjects presented with metabolic syndrome, by mid-pregnancy.



**Figure 4.1** Prevalence of metabolic disorders during mid-pregnancy. Data are presented as frequencies and percentages.

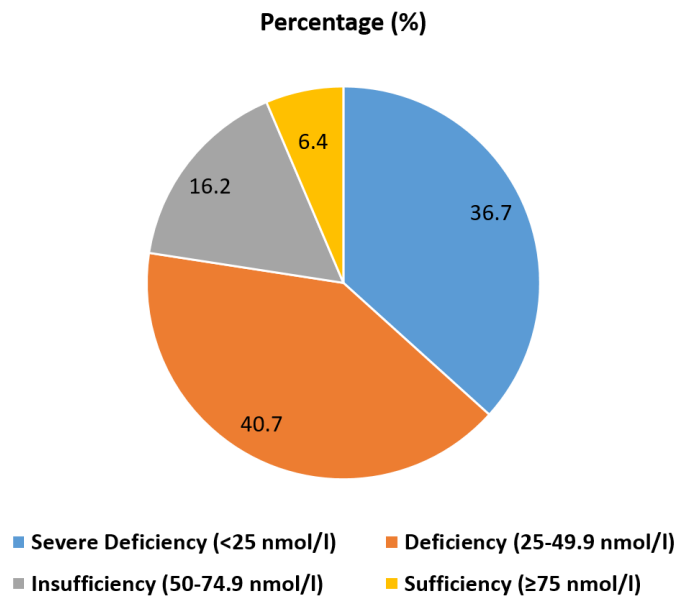
#### ***4.3.2.2 Lifestyle Factors***

The percentage of blood samples collected from the pregnant women during the summer season totalled 51.1%, while 48.9% were collected during the winter. Sun exposure was reported by 22.3% of the participants, and 30.2 % were exposed to the sun at noon time. The subjects who worked exclusively indoors comprised 92.9% of the overall sample. With regard to covering the body with clothing, 30.9% reported full body coverage, whilst only 5.4% of the participants used sunscreen lotion for UV protection.

Estimated vitamin D and calcium intake at mid-pregnancy was noted in the subjects as 149.5 IU/day (67.1-342.7) and 222.8 mg/day (64.3-673.5), respectively. These figures indicate that 2.7% of the subjects were ingesting more than the daily recommended amount for vitamin D, and 12.2% for calcium. In addition, 55% (111/297) reported taking multivitamin supplements by mid-pregnancy. The median sedentary time and low-intensity physical activity/walking reported by the subjects in mid-pregnancy amounted to 948 minutes/week (600-1500), and 230 minutes/week (75-600), respectively.

#### **4.3.3 Vitamin D Status at Mid-pregnancy**

The categorisation of 25(OH)D levels mid-gestation resulted in 36.7% (109/297) of participants having severe vitamin D deficiency; 40.7% (121/297) with vitamin D deficiency; 16.2% with vitamin D insufficiency; and 6.4% (19/297) with sufficiency (**Figure 4.2**). The median circulating 25(OH)D level in the subjects, consisted of 30.5 nmol/L (20.0-48.8).



**Figure 4.2** Vitamin D status at mid-pregnancy

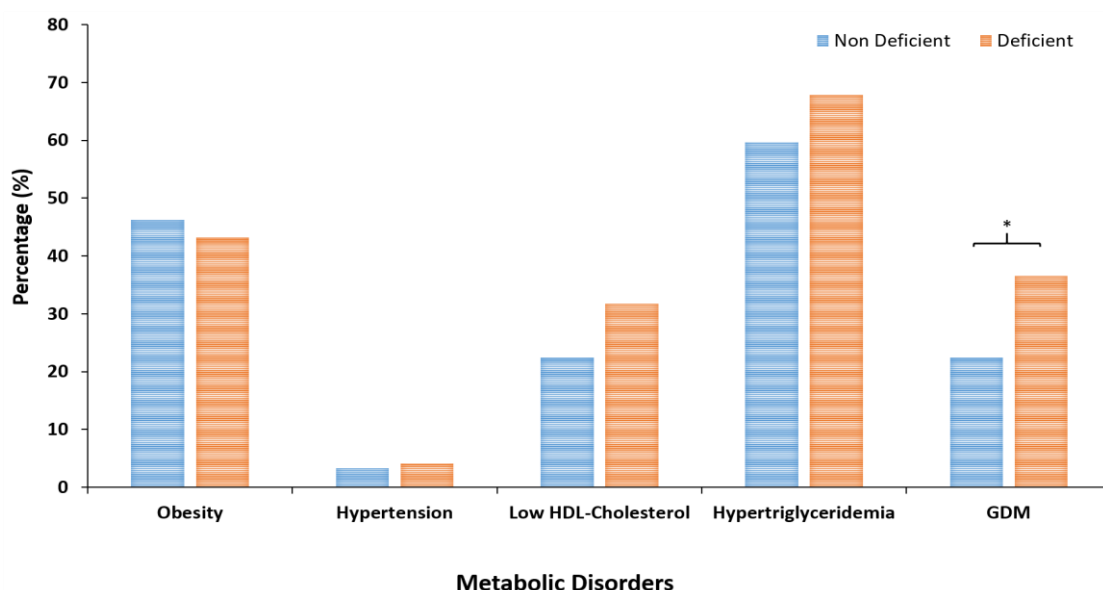
#### ***4.3.3.1 Anthropometric and Biochemical Characteristics in Relation to Vitamin D Status***

By mid-pregnancy, no significant differences were observed in BMI, GWG, body fat percentage, or blood pressure, between the deficient and non-deficient groups (**Table 4.2**). The median 25(OH)D levels in the deficient group was 25.8 nmol/L (17.4-35.1), compared with 61.6 nmol/L (55.4-78.4) in the non-deficient group. Phosphorus levels were lower ( $p = 0.049$ ), and alkaline phosphatase levels were significantly higher ( $p < 0.001$ ) in the vitamin D deficient group, compared to the non-deficient group. In addition, 25(OH)D levels at mid-pregnancy positively correlated with phosphorus ( $r = 0.22$ ,  $p > 0.001$ ) and negatively correlated with alkaline phosphatase ( $r = -0.31$ ,  $p < 0.001$ ). Conversely, TG was significantly higher in the deficient group, compared to the non-deficient group, even after adjusting for age and BMI ( $p = 0.004$ ). GDM markers and insulin are presented in Chapter 6. The presence of characteristics indicating metabolic syndrome and GDM are shown in **Figure 4.3**, and these will be discussed in detail in Chapter 6.

**Table 4.2 Anthropometrics and biochemical characteristics, in relation to vitamin D status at mid-pregnancy**

Parameter	All (N=297)	Non-Deficient (N = 67)	Deficient (N = 230)	P- value	P- value*
<b>Anthropometric Parameters</b>					
Maternal age (years)	28.9 ± 5.3	29.2 ± 5.0	28.8 ± 5.5	0.634	---
<b>Gestational age (in Weeks)</b>	26.3 ± 3.2	25.6 ± 4.0	26.5 ± 2.9	0.040	<b>0.046</b>
Pre-pregnancy BMI (kg/m <sup>2</sup> )	27.0 ± 6.0	26.9 ± 5.5	26.9 ± 6.1	0.918	0.798
BMI (kg/m <sup>2</sup> ) at 2 <sup>nd</sup> visit	30 ± 6.0	30.1 ± 5.4	30.0 ± 6.2	0.882	0.942
Gestational weight gain (GWG) (kg)	7.2 ± 4.3	7.3 ± 4.0	7.1 ± 4.4	0.762	0.675
Waist circumference (cm)	104.9 ± 12.9	104.5 ± 13.0	105.1 ± 12.9	0.736	0.793
Hip circumference (cm)	113.4 ± 11.3	114.5 ± 11.2	113.1 ± 11.3	0.372	0.235
Waist-hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.073	0.095
Systolic BP (mm Hg)	112 ± 12.5	111.1 ± 13.1	112.2 ± 12.3	0.530	0.623
Diastolic BP (mm Hg)	67.9 ± 10.6	67.5 ± 12.6	68.0 ± 10.0	0.790	0.715
Body fat (%)	37.2 ± 3.3	37.3 ± 3.3	37.1 ± 3.3	0.620	0.600
Δ Body fat (%)	10.2 ± 25.1	9.7 ± 16.9	10.3 ± 27.1	0.846	0.920
<b>Biochemical Parameters</b>					
<b>Vitamin D (nmol/L) #</b>	30.5 (20.0 – 48.8)	61.6 (55.4 – 78.4)	25.8 (17.4 – 35.1)	<0.001	<b>&lt;0.001</b>
Corrected calcium (mmol/L)	2.2 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	0.707	0.686
<b>Phosphorus (mmol/L)</b>	1.3 ± 0.5	1.4 ± 0.5	1.3 ± 0.4	0.061	<b>0.049</b>
<b>Alkaline phosphatase (mmol/L)</b>	10.6 ± 4	8.6 ± 2.3	11.2 ± 4.2	<0.001	<b>&lt;0.001</b>
Creatinine (μmol/L)	58.4 ± 16.9	58.4 ± 17.7	58.3 ± 16.8	0.983	0.991
Total cholesterol (mmol/L)	4.6 ± 1.8	6.5 ± 1.2	6.7 ± 1.4	0.239	0.220
HDL-cholesterol (mmol/L)	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	0.577	0.490
Total cholesterol-HDL Ratio	4.6 ± 1.8	4.3 ± 1.5	4.7 ± 1.9	0.090	0.107
LDL-cholesterol (mmol/L)	4.1 ± 1.1	4.0 ± 1.0	4.1 ± 1.1	0.562	0.476
<b>Triglycerides (mmol/L)</b>	2.1 ± 0.7	1.9 ± 0.6	2.1 ± 0.8	0.001	<b>0.004</b>

**Note:** Data presented as a mean and standard deviation for normal variables, while median, first and third quartiles are presented for non-normally distributed variables. # indicates non-normally distributed variables; the p-value for mean differences was obtained from an independent sample t-test for normal variables, and a Mann-Whitney U test for non-normally distributed variables, while \* indicates p-values adjusted for age and BMI on the 1<sup>st</sup> visit through an ANCOVA.



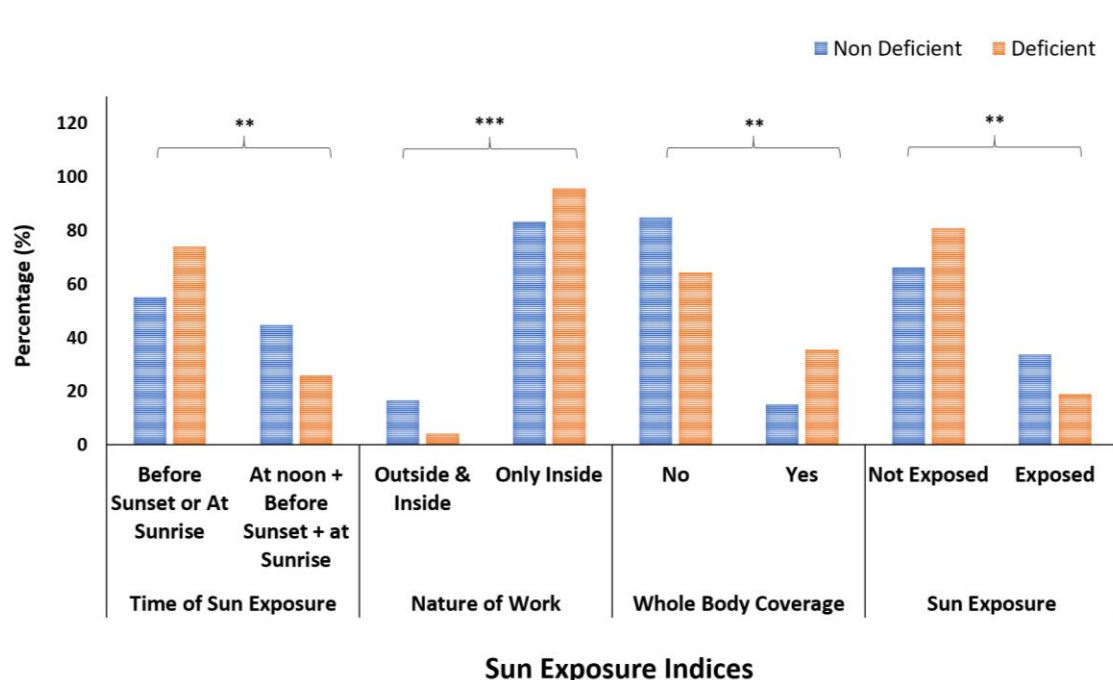
**Figure 4.3** Presence of metabolic disorders in relation to vitamin D status. The values show the percentage of women with different metabolic disorders at mid-pregnancy, in both the vitamin D-deficient and non-deficient groups. P-values: \* denotes  $p < 0.05$ , using the Pearson's chi-square test.

#### 4.3.3.2 Influence of Lifestyle on Vitamin D Status

The subjects with no 25(OH)D deficiency were found to experience a higher rate of sun exposure, compared with the deficient group (33.8% vs. 19%,  $p = 0.011$ ). Therefore, a greater percentage of the participants who spent most of their working lives indoors were found to be deficient, compared to their non-deficient counterparts (95.7% vs. 83.3%,  $p < 0.001$ ). It was similarly significant that more subjects who covered their entire body with clothing were found to be deficient compared with the non-deficient group (35.5% vs. 15.1%,  $p = 0.005$ ). Moreover, the percentage of women exposed to the sun at noon (44.8%) was significantly higher in the non-deficient group, compared to the 26% in the deficient group ( $p = 0.006$ ). The use of sunscreen was represented by 7.5% of the non-deficient group, compared with 4.8% of the deficient group, but this was not statistically significant ( $p = 0.392$ ). Seasonal variations in sun exposure were statistically not different between the groups ( $p = 0.962$ ), with 51.2% of the samples collected during the summer being vitamin D-deficient, compared with 50.8% being classed as non-deficient. Whereas in winter, 48.4% were noted as deficient, compared

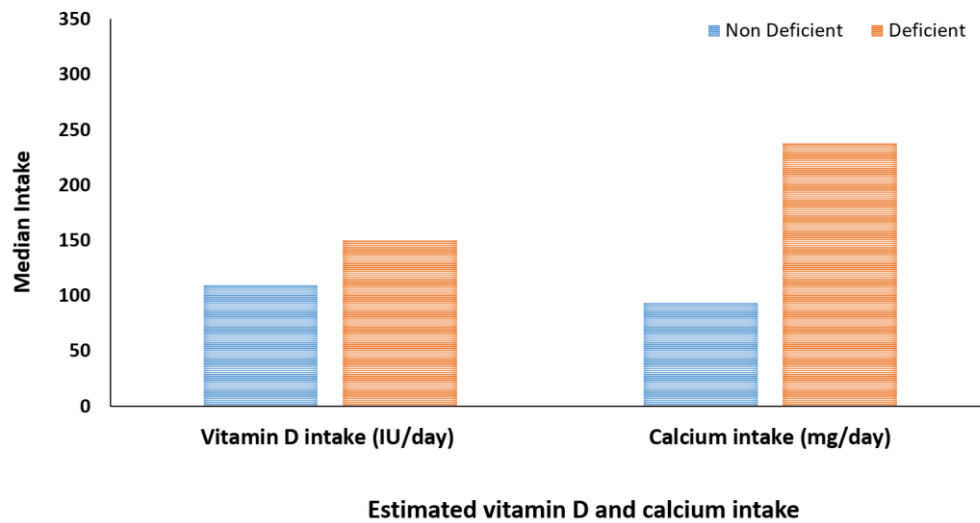


with 49.2% in the non- deficient group. Data on sun exposure variables are presented in **Figure 4.4**.

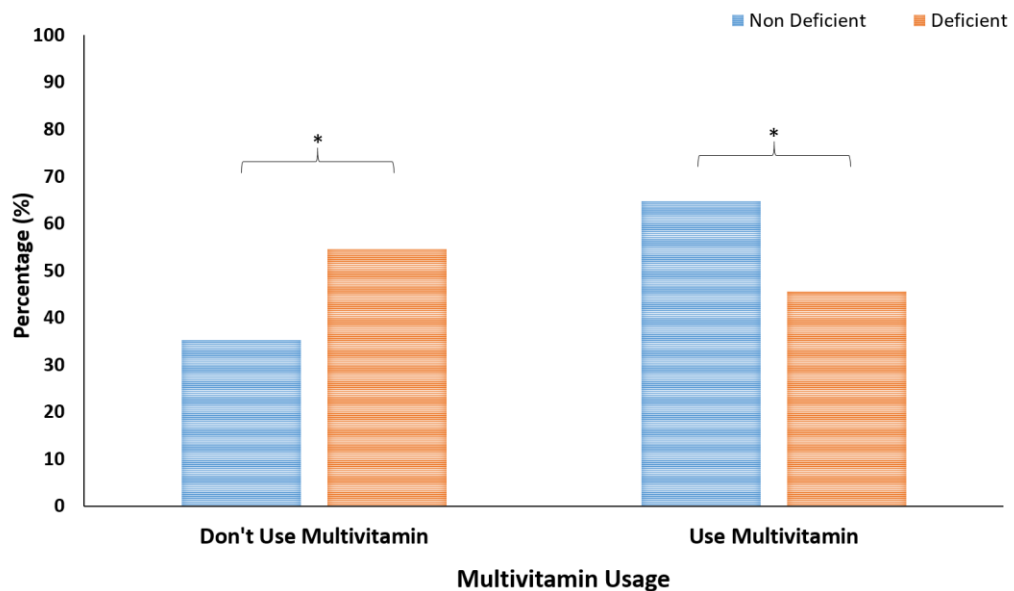


**Figure 4.4** Comparison of the extent of sunlight exposure between the deficient and non-deficient groups, during the second trimester of pregnancy. The values show the percentage of women with different sun exposure indices, in both the vitamin D-deficient and non-deficient groups. P-values: \*\* denotes  $p < 0.01$  and \*\*\* denotes  $p < 0.001$ , using the Pearson's chi-square test.

Estimated vitamin D and calcium intake failed to show any differences between the deficient and non-deficient groups (**Figure 4.5**). Interestingly, multivitamin intake was significantly lower in the 25(OH)D-deficient women (45.5%) by mid-pregnancy, compared with the non-deficient group (64.8%) ( $p = 0.014$ ) (**Figure 4.5**).

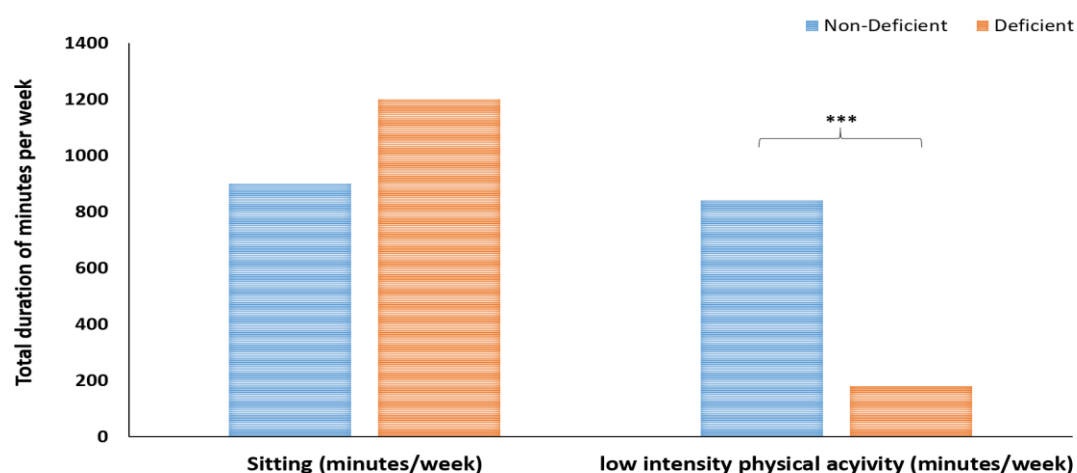


**Figure 4.5** Estimated vitamin D and calcium intake, according to vitamin D status mid-pregnancy. The values show the median intake of vitamin D and calcium from food among both the vitamin D-deficient and non-deficient women at mid-pregnancy, after adjusting for age and BMI. The Mann-Whitney U Test was used.



**Figure 4.6** Intake of multivitamin supplements in relation to vitamin D status at mid-pregnancy. The values show the percentage of multivitamin usage for both deficient and non-deficient women in mid-pregnancy. P-values: \* denotes  $p < 0.05$ . Pearson chi-square test was used.

Regarding physical activity, the median number of minutes for women in the non-deficient vitamin D group who engaged in more low-intensity physical activity/walking than was reported by the deficient group, even after adjusting for age and BMI (840 vs. 180 minutes/week,  $p < 0.001$ ). Vitamin D levels at mid-pregnancy positively correlated with low-intensity physical activity ( $r = 0.43$ ,  $p < 0.001$ ). Furthermore, the women found to be deficient in 25(OH)D, demonstrated a higher trend for remaining sedentary, compared with the non-deficient women, but this was not statistically significant (1200 vs. 900 minutes/week,  $p = 0.113$ ) (**Figure 4.7**).



**Figure 4.7** Comparison of physical activity between the deficient and non-deficient vitamin D groups at mid-pregnancy. The values the median sedentary and physical activity in minutes per week for both the vitamin D-deficient and non-deficient women mid-pregnancy, after adjusting for age and BMI. P-values: \*\*\* denotes  $p < 0.001$ . The Mann-Whitney U Test was used.

#### 4.3.4 Predictors of Vitamin D Deficiency at Mid-pregnancy

Multivariate logistic regression analysis was used to explore significant predictors of vitamin D deficiency. After adjusting for possible confounding factors, the presence of vitamin D deficiency in early pregnancy (OR 4.35 95% CI 1.09-17.36,  $p = 0.037$ ), and working indoors (OR 13.23 95% CI 1.38-126.52,  $p = 0.025$ ) were found to be predictors of vitamin D at mid-pregnancy. In addition, the use of multivitamin supplementation at mid-pregnancy (OR 0.33 95% CI 0.12-0.97,  $p = 0.043$ ), and a greater amount of low-intensity physical activity at mid-pregnancy (OR 0.15 95% CI 0.05-0.45,  $p = 0.001$ ), were found to confer some degree of protection against vitamin D deficiency, during this period.

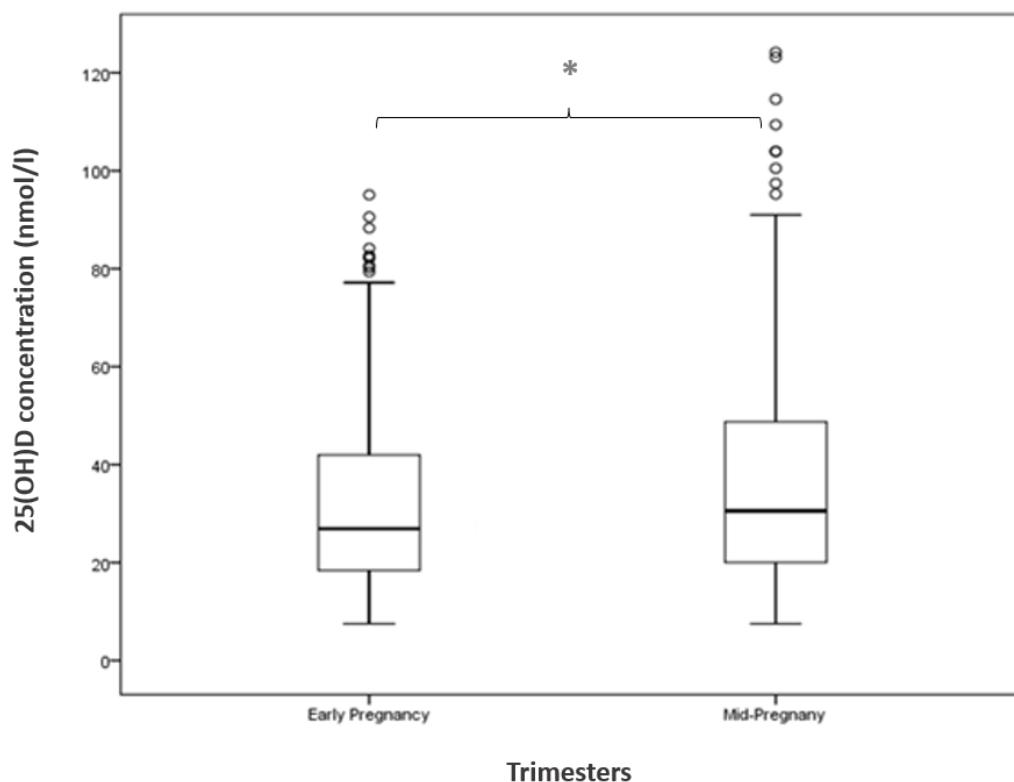
**Table 4.3 Predictors of vitamin D deficiency among the subjects' mid-pregnancy**

Parameters	Unadjusted		Model 1		Model 2	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (years)	0.99 (0.94 – 1.04)	0.633	1.00 (0.94 – 1.05)	0.849	0.99 (0.88 – 1.12)	0.948
Gestational age (weeks)	1.08 (1.00 – 1.17)	0.058	1.07 (0.98 – 1.16)	0.115	0.96 (0.76 – 1.21)	0.697
Pre-pregnancy BMI (kg/m <sup>2</sup> )	1.00 (0.95 – 1.04)	0.918	1.00 (0.95 – 1.05)	0.969	1.07 (0.96 – 1.19)	0.211
Obesity at 2 <sup>nd</sup> visit	0.88 (0.51 - 1.53)	0.660	0.71 (0.28 – 1.81)	0.471	0.46 (0.08 – 2.67)	0.383
Gestational weight gain (GWG) (excessive)	0.83 (0.47 - 1.45)	0.506	0.84 (0.46 - 1.54)	0.565	1.50 (0.40 – 5.53)	0.547
Body fat %	0.98 (0.90 - 1.07)	0.620	0.95 (0.87 - 1.05)	0.318	0.97 (0.84 - 1.13)	0.736
Δ Body fat (%)	1.00 (0.99 – 1.01)	0.846	1.00 (0.99 – 1.01)	0.967	1.00 (0.97 – 1.04)	0.939
Blood sample collection (summer) at 2 <sup>nd</sup> visit	1.01 (0.57 - 1.79)	0.962	0.91 (0.50 - 1.64)	0.762	0.81 (0.28 – 2.34)	0.698
Sun exposure by 1 <sup>st</sup> visit	0.55 (0.30 – 1.01)	0.053	0.54 (0.29 – 1.00)	0.050	0.71 (0.24 – 2.11)	0.533
Sun exposure by 2 <sup>nd</sup> visit	0.46 (0.25 - 0.85)	0.011	0.44 (0.24 - 0.81)	0.008	0.92 (0.29 – 2.9)	0.884
Time of exposure (noon) by 2 <sup>nd</sup> visit	0.43 (0.24 - 0.79)	0.007	0.43 (0.23 – 0.82)	0.010	0.39 (0.12 - 1.23)	0.109
<b>Nature of Work (Indoor) by 2<sup>nd</sup> visit</b>	4.40 (1.78 - 10.89)	0.001	4.37 (1.62 – 11.80)	0.004	13.23 (1.38 – 126.52)	<b>0.025</b>
Coverage with clothing (whole body) by 2 <sup>nd</sup> visit	3.10 (1.38 - 6.97)	0.006	2.54 (1.09 – 5.90)	0.030	2.50 (0.70 – 8.90)	0.156
Coverage with clothing (whole body) by 1 <sup>st</sup> visit	2.53 (1.30 – 4.90)	0.006	2.09 (1.06 – 4.14)	0.034	1.62 (0.59 – 4.41)	0.585
HbA1c at 2 <sup>nd</sup> visit	2.10 (1.10 - 3.99)	0.023	2.09 (1.05 - 4.17)	0.036	2.36 (0.67 - 8.34)	0.181
Fasting glucose (mmol/l) at 2 <sup>nd</sup> visit	1.45 (1.01 - 2.08)	0.026	1.48 (1.01 – 2.18)	0.046	0.78 (0.43 - 1.41)	0.783
<b>Vitamin D Deficiency at 1<sup>st</sup> visit</b>	3.53 (1.85 - 6.72)	< 0.001	3.40 (1.72 – 6.73)	< 0.001	4.35 (1.09 - 17.36)	<b>0.037</b>
Cholesterol-HDL ratio at 2 <sup>nd</sup> visit > 3.5	1.16 (0.64 – 2.09)	0.625	1.43 (0.77 – 2.67)	0.261	1.49 (0.44 – 5.07)	0.526
Low HDL at 2 <sup>nd</sup> visit (>1.03mmol/l)	1.61 (0.85 – 3.05)	0.142	1.98 (0.99 – 3.96)	0.052	2.69 (0.69 – 10.54)	0.156
Hypertriglyceridemia at 2 <sup>nd</sup> visit	1.42 (0.81 - 2.49)	0.218	1.50 (0.83 - 2.71)	0.185	0.55 (0.19 - 1.60)	0.270
Metabolic syndrome at 2 <sup>nd</sup> visit	1.54 (0.70 - 3.37)	0.280	1.77 (0.73 - 4.32)	0.207	2.68 (0.42 – 17.19)	0.298
Gestational diabetes mellitus (GDM)	2.00 (1.06 – 3.76)	0.033	1.93 (0.99 – 3.75)	0.054	1.04 (0.32 – 3.33)	0.953
Hypertension (HTN) at 2 <sup>nd</sup> visit	1.28 (0.27 – 6.07)	0.759	0.92 (0.19 – 4.50)	0.914	2.39 (0.14 – 40.52)	0.546
Vitamin D intake by 1 <sup>st</sup> visit (> 600 IU/day)	0.29 (0.06 – 1.46)	0.133	0.35 (0.07 – 1.86)	0.216	0.28 (0.001 – 99.96)	0.672
Vitamin D intake by 2 <sup>nd</sup> visit (> 600 IU/day)	0.74 (0.14 - 3.91)	0.720	0.87 (0.16 - 4.80)	0.874	0.18 (0.02 - 1.83)	0.146
Calcium intake by 2 <sup>nd</sup> visit ( > 1000 mg/day)	0.48 (0.20 - 1.11)	0.080	0.54 (0.22 - 1.30)	0.169	0.29 (0.07 - 1.16)	0.079
Use of multivitamins by 1 <sup>st</sup> visit	2.00 (0.90 – 4.37)	0.088	1.66 (0.74 – 3.71)	0.221	1.74 (0.33 – 9.18)	0.513
<b>Use of Multivitamins by 2<sup>nd</sup> visit</b>	0.45 (0.24 - 0.86)	0.015	0.44 (0.23 - 0.85)	0.014	0.33 (0.12 - 0.97)	<b>0.043</b>
Low intensity PA (≥ 210 minutes/week) by 1 <sup>st</sup> visit	0.89 (0.51 – 1.54)	0.670	0.92 (0.51 – 1.66)	0.788	0.65 (0.26 – 1.60)	0.344
<b>Low Intensity PA (≥ 210 minutes/week) by 2<sup>nd</sup> visit</b>	0.20 (0.10 – 0.39)	< 0.001	0.18 (0.09 – 0.37)	< 0.001	0.15 (0.05 – 0.45)	<b>0.001</b>

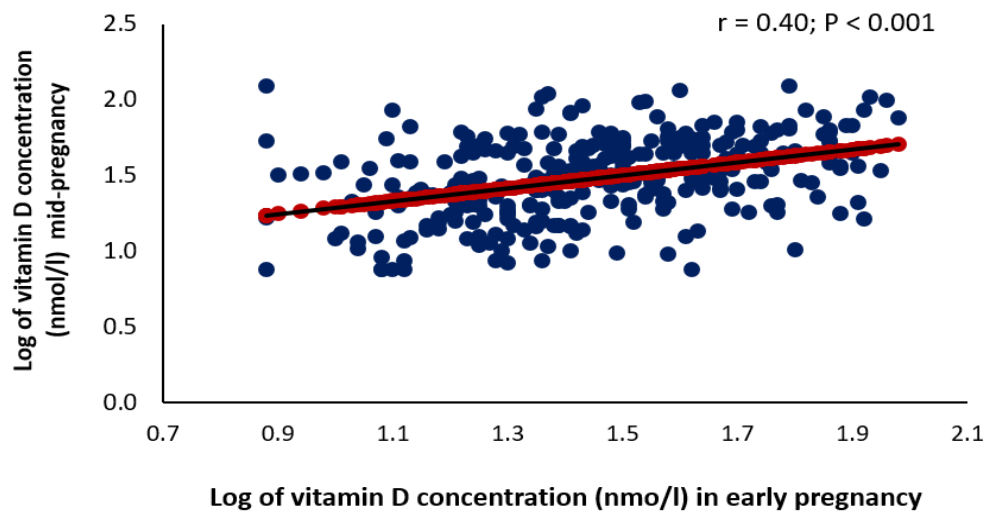
**Note:** Data are presented as frequency (%) for categorical variables, while mean (SD) and median (1<sup>st</sup> quartile-3<sup>rd</sup> quartile) are presented for normally distributed and non-normally distributed quantitative variables, respectively. Odds ratio (OR) and 95% CI for OR were obtained using logistic regression analysis, taking vitamin D deficiency (< 50 nmol/L) as a dependent variable against potential independent risk factors; Model 1 is for age, BMI, and sun exposure. Model 2 is adjustment 1+ gestational weight gain, parity, season (summer), vitamin D and multivitamin intake, physical activity, education, employment, place of residence (north Riyadh), and coverage with clothing. The categorical variables, including GDM, HTN, sun exposure and multivitamin supplementation are presented as 'yes/no'. Other categorical values, such as blood sample collection in summer vs. winter; time of sun exposure (noon alone plus either sunset or sunrise, or both vs. sunrise or sunset or both), working indoors vs. both indoors and outdoors, and whole body coverage vs. partial coverage with clothing.

### 4.3.5 Alterations in Vitamin D Status During Pregnancy

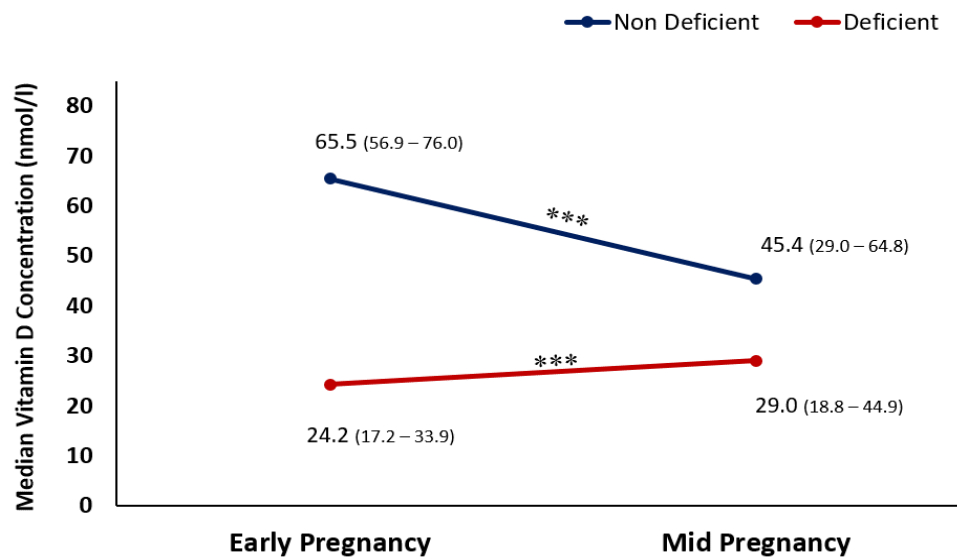
During early gestation, 83% (247/297) of the subjects displayed vitamin D deficiency, which reduced to 77% (230/297) by mid-pregnancy. This highlights the fact that participant's vitamin D levels slightly increased from early to mid-pregnancy, revealing that the increase in vitamin D from early to mid-pregnancy [26.9 (18.2-42.0) vs. 30.5 (20.0-48.8) nmol/L,  $p = 0.038$ ] remained significant, even after adjusting for mid-pregnancy multivitamin intake, physical activity, estimated vitamin D-intake, sun exposure, season, and gestational age ( $p < 0.001$ ) (**Figure 4.8**). Vitamin D levels at mid-pregnancy also positively correlated with vitamin D during early pregnancy ( $r = 0.40$ ,  $p < 0.001$ ) (**Figure 4.9**). Furthermore, vitamin D levels significantly decreased from early to mid-pregnancy in the non-deficient group, but significantly increased in the deficient group (**Figure 3.10**).



**Figure 4.8** 25(OH)D status from early to mid-pregnancy. The values show the median vitamin D from early to mid-pregnancy, using the Wilcoxon signed rank test. P-value: \* denotes  $p < 0.05$ .



**Figure 4.9** Correlation between vitamin D (nmol/L) at mid-pregnancy vs. vitamin D (nmol/L) in early pregnancy.



**Figure 4.10** Median vitamin D concentrations (nmol/L) in early and mid-pregnancy, in both deficient and non deficient groups, according to the Wilcoxon signed rank test. P-value: \*\*\* denotes  $p < 0.001$ .

### 4.3.6 Comparison Between Variables During Early and Mid-term Pregnancy

As expected, body fat percentages significantly increased from early to mid-pregnancy ( $p < 0.001$ ). Interestingly, the data revealed that the increase in body fat percentage was greater from early to mid-pregnancy ( $33.4 \pm 5.8\%$  to  $37.0 \pm 3.2\%$ ,  $p < 0.001$ ) in the vitamin D-deficient women, compared with the non-deficient group ( $35.7 \pm 5.7\%$  to  $38 \pm 3.4\%$ ,  $p = 0.002$ ) (**Table 4.4**).

In the biochemical profile, the total cholesterol ( $5.2 \pm 1$  to  $6.6 \pm 1.4$  mmol/L,  $p < 0.001$ ), HDL-cholesterol ( $1.3 \pm 0.4$  to  $1.6 \pm 0.5$  mmol/L,  $p < 0.001$ ), LDL-cholesterol ( $3.2 \pm 0.7$  to  $4.0 \pm 1.1$  mmol/l,  $p < 0.001$ ), and the total cholesterol/HDL ratio ( $4.1 \pm 1.1$  to  $4.6 \pm 1.8$  mmol/L,  $p < 0.001$ ), all significantly increased from early to mid-pregnancy, in both groups (**Table 4.4**). Phosphorus ( $1.2 \pm 0.4$  to  $1.3 \pm 0.5$  mmol/L,  $p < 0.001$ ), and alkaline phosphatase ( $9.7 \pm 2.9$  to  $10.4 \pm 4.0$  mmol/L,  $p < 0.01$ ) also increased significantly from early to mid-pregnancy, among all the participants. However, phosphorus and alkaline only increased significantly in the deficient group, but did not show any difference in the non-deficient group (**Table 4.4**). Moreover, corrected calcium levels did not show any statistical difference from early to mid-pregnancy ( $2.2 \pm 0.2$  to  $2.2 \pm 0.2$  mmol/L,  $p = 0.345$ ).

Sun exposure variables did not change from early to mid-pregnancy. Estimated vitamin D [108.5 (63.1-265.5) to 149.5 (67.1-344.5),  $p < 0.001$ ], and calcium intake [163.4 (65.6-596.4) to 212.4 (64.2-673.5),  $p < 0.001$ ] showed a significant increase from early to mid-pregnancy, across the entire sample. Moreover, estimated vitamin D and calcium intake significantly increased in the deficient group from early to mid-pregnancy, while the non-deficient group did not display any significant differences (**Table 4.4**). Multivitamin intake increased from 19.4% in early pregnancy to 47.4% mid-pregnancy ( $p < 0.001$ ), which was significant in both the deficient and non-deficient groups, although this significance was more pronounced in the deficient group ( $p < 0.001$ ) than among the non-deficient group ( $p = 0.021$ ) (**Table 4.4**).

Sedentary time and low-intensity physical activity/walking did not differ between the two groups from early to mid-pregnancy [205 minutes/week (75-600) vs. 230 minutes/week (75-637.5),  $p = 0.162$ ] and [900 (600-1500) vs. 948.2 (600-1500),  $p = 0.135$ ], respectively. Meanwhile, low-intensity physical activity significantly decreased in the non-deficient group ( $p = 0.01$ ), but significantly increased in the deficient group ( $p = 0.002$ ). However, sedentary time in minutes/week increased significantly in the non-deficient group ( $p = 0.038$ ), but only showed an increasing trend among the deficient women, although this was not statistically significant ( $p = 0.466$ ) (**Table 4.4**).



**Table 4.4 Comparison of early to mid-pregnancy based on vitamin D status**

Parameters	Non-Deficient (N=50)			Deficient (N=247)		
	Visit 1	Visit 2	P-value	Visit 1	Visit 2	P-value
Systolic BP	116.8 ± 13.2	111.2 ± 15.6	0.014	113.9 ± 13.6	112 ± 12	0.039
Diastolic BP	69.4 ± 11.2	70.1 ± 12.3	0.698	67.6 ± 9.5	67.2 ± 10	0.603
Body fat (%)	35.7 ± 5.7	38 ± 3.4	<b>0.002</b>	33.4 ± 5.8	37.0 ± 3.2	<b>&lt; 0.001</b>
<b>Biochemical Parameters</b>						
Vitamin D (nmol/L) #	65.5 (56.9 – 76.0)	45.3 (29.0 – 64.8)	<b>&lt; 0.001</b>	24.2 (17.2 – 33.9)	29.0 (18.8 – 44.9)	<b>&lt; 0.001</b>
Total cholesterol (mmol/L)	5.2 ± 0.8	6.6 ± 1.3	<b>&lt; 0.001</b>	5.2 ± 1	6.6 ± 1.4	<b>&lt; 0.001</b>
HDL-cholesterol (mmol/L)	1.4 ± 0.3	1.6 ± 0.6	<b>0.036</b>	1.3 ± 0.4	1.6 ± 0.5	<b>&lt; 0.001</b>
Cholesterol-HDL ratio	3.7 ± 0.7	4.7 ± 2	<b>0.002</b>	4.1 ± 1.2	4.6 ± 1.8	<b>&lt; 0.001</b>
LDL-Cholesterol (mmol/L)	3.1 ± 0.6	4.1 ± 1	<b>&lt; 0.001</b>	3.2 ± 0.8	4.0 ± 1.1	<b>&lt; 0.001</b>
Corrected calcium (mmol/L)	2.2 ± 0.2	2.3 ± 0.2	0.668	2.2 ± 0.2	2.2 ± 0.2	0.402
Phosphorus (mmol/L)	1.3 ± 0.5	1.4 ± 0.4	0.410	1.1 ± 0.4	1.3 ± 0.5	<b>&lt; 0.001</b>
Creatinine (μmol/L)	60.5 ± 18.7	63.3 ± 15.9	0.480	56.9 ± 17.6	56.5 ± 17.1	0.837
Glucose (mmol/L) #	4.8 (4.3 – 5.2)	4.3 (3.9 – 4.6)	<b>&lt; 0.001</b>	4.8 (4.4 – 5.4)	4.5 (4.0 – 5.1)	<b>&lt; 0.001</b>
Insulin (uU/mL) #	10.5 (4.8 – 20.3)	9.7 (5.3 – 13.5)	0.372	8.3 (4.6 – 18.6)	7.6 (5.0 – 12.1)	<b>0.009</b>
Alkaline Phosphate	9.7 ± 1.9	9.9 ± 4.4	0.866	9.6 ± 3.09	10.5 ± 3.9	<b>0.005</b>
<b>Diet</b>						
Vitamin D intake (IU)/day #	91.0 (58.2 - 331.2)	163.0 (58.7 - 373.8)	0.119	108.5 (64.7 - 251.5)	147.2 (67.5 - 331.2)	<b>&lt;0.001</b>
Calcium intake (mg)/day #	137.2 (57.7 - 613.0)	162.2 (57.4 - 650.9)	0.334	163.4 (66.2 - 596.4)	222.8 (65.6 - 686.7)	<b>&lt;0.001</b>
Multivitamin intake	7 (22.6)	15(48.4)	<b>0.021</b>	23 (15.8)	66 (45.2)	<b>&lt;0.001</b>
<b>Physical Activity</b>						
Sitting (minutes/week) #	600.0 (450 – 1500)	911.3 (600 – 1500)	<b>0.038</b>	900.0 (600 – 1500)	1147.4 (600 – 15000)	0.466
Low intensity (minutes/week) #	840.0 (311.3 – 1500)	435.0 (103.7 – 1000)	<b>0.014</b>	180.0 (62.5 – 420.0)	210.0 (70 – 594.4)	<b>0.002</b>

**Note:** Data are presented as the mean and standard deviation for normal variables, while median, first and third quartiles are presented for non-normally distributed variables. # indicates non-normal variables; the p-value for mean differences was obtained using a paired sample t-test for normally distributed variables, and a Wilcoxon signed rank test for non-normally distributed variables.

## 4.4 Discussion

In the present study, it must be borne in mind that approximately one-third of the sample failed to attend the mid-pregnancy visit. Those who did not attend were significantly younger, with a better metabolic profile and higher vitamin D levels. However, they also demonstrated significantly less calcium and vitamin D intake, reflecting an unhealthy diet. Moreover, the participants who withdrew before the second visit were more likely to reside in West Riyadh, which is associated with a lower level of education, compared to other parts of the city. In addition, a family history of obesity and diabetes was noted for the participants who failed to attend the second visit, and they were more likely to have had a previous miscarriage. However, the overall better state of health amongst these women withdrawing from the study meant that the figures derived at mid-pregnancy were not over-estimated.

The present finding demonstrates that the prevalence of 25(OH)D deficiency ( $< 50$  nmol/L) in the second trimester of pregnancy amounted to about three quarters (77.4%) of the women studied. A higher percentage (86.4%) of vitamin D deficiency in late pregnancy has also been reported in KSA (Al-Shaikh et al., 2016). The above study, along with the findings from the current study, observed an alarmingly high prevalence of vitamin D deficiency in Saudi Arabia. However, these observations may not represent the actual status of vitamin D deficiency across the entire Kingdom, because they were made exclusively in Riyadh, which is located in the centre of the country. Notwithstanding the above, other studies, have similarly stated a prevalence of vitamin D deficiency at mid-pregnancy, amounting to 78.7% in China and 74% in India (Sahu et al., 2009; Xiao et al., 2015). The situation in the USA appears to be slightly better, with 34.8% of pregnant women found to suffer from hypovitaminosis D ( $< 37.5$  nmol/L) (Gernand et al., 2013). Similarly, in Qatar, 48.4% of pregnant women studied were noted as being deficient in vitamin D, although the above study also reported that 32.5% of the participants consumed vitamin D supplements (Bener et al., 2013). Nevertheless, vitamin D deficiency still appears to be especially high among pregnant Saudi women, even if this is a general global issue, particularly among pregnant women.

Despite the fact that the majority of the pregnant women in the present study were determined to be deficient in vitamin D by their second trimester, concentrations of the vitamin were significantly higher than in the first trimester. Similar observations of a progressive increase in blood vitamin D status have previously been reported, with significantly higher 25(OH)D status during the second trimester, compared to the first trimester of pregnancy (Charatcharoenwitthaya et al., 2013). Other studies have also reported that serum vitamin D levels can demonstrate an insignificant increase from the first to the second trimester (Park et al., 2014; Choi et al., 2015; Lundqvist et al., 2016). However, compared to the first trimester, a significant increase in vitamin D levels during the third trimester is in keeping with the results of the present study; suggesting a progressive increase in serum vitamin D levels as the pregnancy advances (Bodnar et al., 2007b; Ginde et al., 2010; Vandevijvere et al., 2012; Charatcharoenwitthaya et al., 2013; Choi et al., 2015; Bärebring et al., 2016; Lundqvist et al., 2016). A decreasing trend, or absence of any change, in serum vitamin D levels with advancing pregnancy have also been reported in a number of other papers (Ritchie et al., 1998; Marwaha et al., 2011; Fernández-Alonso et al., 2012; Zhang et al., 2014; Jafarzadeh et al., 2015; Moon et al., 2015). Although, it is crucial to note that no study has observed static trends in vitamin D concentration during pregnancy, if corrected for seasonal and lifestyle factors. For example, a recent study corrected for seasonal factors revealed that vitamin D levels tend to increase as pregnancy progresses (Bärebring et al., 2016). Moreover, higher baseline concentrations of vitamin D in early pregnancy are better predictors of higher concentrations during the later stages of gestation (Ginde et al., 2010; Charatcharoenwitthaya et al., 2013; Lundqvist et al., 2016).

Similar to the results described in Chapter 3, seasonal factors were not found to impact vitamin D levels, although sun exposure indices were associated with vitamin D levels at mid-pregnancy. Other studies conducted during mid-pregnancy have yielded findings to support the above (Bener et al., 2013; Parildar et al., 2013; Xiang et al., 2013). Additionally, estimated vitamin D and calcium intake failed to display any significant difference in mid-pregnancy vitamin D concentrations. In general, vitamin

D, calcium, and multivitamin intake in the present study were found to significantly increase as the pregnancies progressed. This may be explained by improved appetite and food cravings during mid-pregnancy, after the first-trimester pregnancy symptoms have diminished (Bayley et al., 2002). Food cravings naturally appear late during early gestation, and peak mid-gestation, before weakening as gestation progresses to full term (Bayley et al., 2002).

In the present study, by mid-pregnancy, multivitamin supplementation was found to confirm a slight increase in vitamin D levels, and hence, could be indicated as having a partial role in enhancing vitamin D levels from early to mid-pregnancy. Numerous studies conducted in the past have reported the impact of multivitamins on vitamin D levels (Bodnar et al., 2007b; Ginde et al., 2010; Charatcharoenwitthaya et al., 2013). For example, an intake of 400 IU of multivitamins daily, may be inadequate for increasing vitamin D concentrations in expectant mothers to  $\geq 50$  nmol/L, especially among high-risk women, such as ethnic minorities and women living at northern latitudes, and for winter pregnancies (Cockburn et al., 1980; Hollis & Wagner, 2004; Ginde et al., 2010). This multivitamin concentration may help to prevent hypovitaminosis D in women with adequate levels of vitamin D, but would be inadequate for resolving insufficiency, if used in isolation (50-75 nmol/L) (Charatcharoenwitthaya et al., 2013). Thus, vitamin D supplementation in higher doses is essential for pregnant women, especially Saudi women, where the majority are already vitamin D-deficient. A daily supplement of 4000 IU/day of vitamin D is considered to be safe, and reasonably adequate for avoiding vitamin D deficiency in pregnancy (Hollis et al., 2011).

Also comparable to Chapter 3, neither BMI nor fat percentage correlated with serum vitamin D levels in the second half of pregnancy, which is supported by other observations made previously (Clifton-Bligh et al., 2008; Farrant et al., 2008; Soheilykhah et al., 2010; Lau et al., 2011; Josefson et al., 2013; Karras et al., 2013; Karlsson et al., 2015; Simões et al., 2016). Conversely, an inverse correlation between BMI and vitamin D levels during the second half of gestation has also been reported (Bodnar et al., 2007b; Soheilykhah et al., 2010; Zuhur et al., 2013; Arnold et al., 2015).

However, similar to the current findings, no such correlations were reported alongside adiposity (body fat content) in the literature. In addition, as with obesity and adiposity, a null relationship was observed for GWG and vitamin D concentration. However, there are controversial and insufficient data to suggest that maternal obesity/adiposity reduces maternal vitamin D concentrations, especially during mid- and late pregnancy (Simões et al., 2016). Possible explanations for the absence of an association between adiposity and maternal vitamin D concentration in previous studies include: (1) no difference between the mean BMI, fat percentage, and GWG in the deficient and non-deficient groups, (2) changes in vitamin D metabolism during pregnancy (Salle et al., 2000; Song et al., 2013), (3) the failure to determine any vitamin D protein ligands (Brannon & Picciano, 2011), and (4) increased total body water content (haemodilution), observed during pregnancy. However, the mechanisms involved have yet to be elucidated.

Also, similar to Chapter 3, physical activity in mid-pregnancy was found to positively correlate with serum vitamin D levels, and also emerged as an independent determinant for predicting 25(OH)D concentration at mid-pregnancy. A positive correlation between physical activity and 25(OH)D concentration during gestation has been described in the past (Wuertz et al., 2013; Moon et al., 2015; Simões et al., 2016). In the present study, physical activity was indeed found to restore vitamin D levels to an extent, but this fell short of optimal restoration, although this could be due to incidental sun exposure, experienced as individuals participate in physical activity. However, there are suggestions that physical activity *per se* has an independent effect on vitamin D levels, a suggestion that is implicated here, as the increase in vitamin D concentration was still significant, even after adjusting for sun exposure. However, this has rarely been investigated during pregnancy in any detail.

During the second trimester, cardiometabolic profiles were associated with vitamin D deficiency, with pronounced significance in TG levels. This confirms the role of vitamin D deficiency in increasing the incidence of metabolic disorders (Boucher, 1998; Barter et al., 2007), but there is limited information regarding the link between

25(OH)D concentration and lipid profiles during pregnancy. A cross-sectional study investigating non-pregnant Canadian female adults, with a high risk of T2DM and obesity, has nevertheless shown that TG decreased as much as 0.14 mmol/L per 1 nmol/L increase in vitamin D (Mansuri et al., 2015). It has been suggested that 25(OH)D may facilitate the removal of lipoprotein particles, by increasing the gene expression of lipoprotein lipase (Vu et al., 1996). Moreover, hyperparathyroidism, secondary to hypovitaminosis D could also decrease the peripheral removal of TG (Cho et al., 2005; Kwon & Lim, 2016).

In the current study, 25(OH)D deficiency in early pregnancy represented an independent risk factor for vitamin D deficiency at mid-pregnancy, after adjusting for confounding factors. In addition, vitamin D deficiency in early pregnancy has been proposed as an independent predictor of 25(OH)D inadequacy in the third trimester of pregnancy (Charatcharoenwitthaya et al., 2013; Choi et al., 2015).

The reason for the slight increase in vitamin D observed from early to mid-pregnancy is partly due to physical activity, as well as vitamin D and multivitamin intake. Other variables include sun exposure, obesity, season (summer), and body fat percentage; did not reveal any association with changes in vitamin D levels from early to mid-pregnancy, at either of the visits. Moreover, it is possible that vitamin D-binding proteins, or genetic factors, play a role in vitamin D increases (Wang et al., 2010).

It was also evident that vitamin D levels behaved differently in the deficient and non-deficient groups. In the deficient group, 25(OH)D increased from early to mid-pregnancy, while in the non-deficient group, vitamin D levels appeared to decrease from early to mid-pregnancy. It can, therefore, be concluded in this study that the deficient group was most influential on the overall population sample, thus showing a trend towards an increase. In this regard, it is possible that the vitamin D-deficient subjects, after being informed of their vitamin D status, increased their multivitamin intake, consumed more vitamin D rich food, and engaged in more physical activity, compared to their non-deficient counterparts.

In conclusion, the strongest predictors of vitamin D status in pregnancy emerged as multivitamin supplementation, working indoors, and physical activity. Some of the disagreement arising between other published literature and the present data may be attributed to different study conditions, methods of vitamin D analysis, varying sample size and demographics, different latitudes and duration of UV exposure, seasonal factors, and lifestyle habits. Other reasons for the changing serum vitamin D concentrations seen in pregnancy, could include altered liver 25-hydroxylase activity, changes in intact PTH levels, increased foetal metabolic activity (Cushard et al., 1972; Sanchez et al., 1997), and the influence of placenta-derived hormones (Kovacs & Kronenberg, 1997; Tobias & Cooper, 2004). Therefore, further research is essential for exploring these factors in Saudi Arabia.

## **Chapter 5**

### **Prevalence and Risk Factors of Gestational Diabetes Mellitus Among Saudi Women**



## 5.1 Introduction

The prevalence of obesity and T2DM is high throughout the world, but particularly in Middle Eastern countries (Al-Daghri et al., 2010; Bahijri et al., 2016). For example, it has been reported that as many as 34.6 million people in the Middle East were diagnosed with T2DM in 2013, and this is expected to rise to 67.9 million people by 2035; an increment of 96% (Guariguata et al., 2014). According to the International Diabetes Federation (IDF), the prevalence of adult T2DM in Saudi Arabia is estimated at 17.4% (IDF, 2015). This is particularly concerning, due to the emerging trends of further disease, beyond T2DM, and the importance of identifying high-risk groups early. Having GDM has been recognised as the strongest predictor of T2DM, with a 7-8 times higher risk of T2DM later in life (Kim et al., 2002; Yang et al., 2009).

The prevalence of GDM ranges from 1-14% of all pregnancies, depending on the diagnostic criteria applied, and the ethnic group involved (ADA, 2004; Jang, 2011; Kim et al., 2012). Data on the overall prevalence of GDM in Saudi Arabia remains limited. Nevertheless, in 2015, one study performed in the Saudi Arabian city named Al-Madina reported a prevalence of 39.4% for GDM, using the IADPSG diagnostic criteria (Alfadhli et al., 2015).

GDM is associated with multiple adverse maternal and neonatal complications (Kjos & Buchanan, 1999; Reece, 2010). For instance, it is known to cause almost 7% of pregnancy-related complications, including hypertension and pre-eclampsia, as well as leading to caesarean section, infection, and polyhydramnios (Kjos & Buchanan, 1999; Di Cianni et al., 2003; Gasim, 2012; Chen et al., 2015). Additionally, GDM is related to long and short term foetal complications (Kjos & Buchanan, 1999; Erem et al., 2003; Gasim, 2012; Alfadhli et al., 2015). Furthermore, mothers with GDM tend to suffer a financial burden that can be around 25% higher, and bear 44-49% greater costs for inpatient care, in comparison with a normal pregnancy (Kolu et al., 2012). Therefore, the timely identification of factors predisposing pregnant women to GDM, not only appears to be cost effective, but may even be critical for avoiding associated morbidity.

A number of pre-pregnancy, or pregnancy-related risk factors, associated with GDM have been identified beyond simple obesity. The pre-pregnancy factors include age (over 35 years old), ethnicity, multiparity, changes in weight between pregnancies, cigarette smoking, family history of T2DM or GDM, previous GDM, history of foetal death, previous macrosomia, and previous caesarean section (Nohira et al., 2006; Russell et al., 2008; Dode & Santos, 2009; Reece et al., 2009; Al-Rowaily & Abolfotouh, 2010; Nankervis et al., 2012). Polycystic ovary syndrome has also been implicated in the later development of IGT and GDM (Noctor & Dunne, 2015). Furthermore, at least 10 genes with specific polymorphisms have been linked with an elevated risk of GDM, particularly the TCF7L2 gene (Zhang et al., 2013). Additionally, high levels of serum  $\gamma$ -glutamyl transferase in the years prior to pregnancy have been identified as an intrinsic factor for the development of GDM (Osorio, 2014).

Pregnancy-related factors of increased risk of GDM, such as high random and fasting glucose levels during pregnancy (Kim et al., 2002), an abnormal OGTT result (Jang, 2011; Noctor & Dunne, 2015), and high HbA1c during early pregnancy, have also been associated with future risk of GDM (Hughes et al., 2014). Furthermore, inadequate insulin secretion following OGTT is implicated in the development of abnormal glucose tolerance, and GDM risk (Buchanan et al., 1998). Moreover, excessive GWG (Hantoushzadeh et al., 2016), unhealthy dietary habits, and physical inactivity are considered to be potential risk factors for the development of GDM (Dode & Santos, 2009; Erem et al., 2015), while lipid profile abnormalities in early pregnancy are also associated with GDM future development (Enquobahrie et al., 2005; Li et al., 2015).

The aim of the present study was to estimate the prevalence of GDM among pregnant Saudi women, using the IADSPG criteria. Although the risk factors of GDM in Saudi communities may vary from those of other populations, it is important to understand the key influences, and also the impact of gestational age, on classical and non-classical factors influencing the onset of GDM.

## **5.2 Study Design and Methods**

### **5.2.1 Study Design and Population**

This was a prospective observational study of 297 pregnant women with a mean age  $28.9 \pm 5.3$  years, followed from the first to the second trimester of pregnancy. The inclusion and exclusion criteria were applied as explained in Chapter 2.

### **5.2.2 Data Collection**

Socio-economic data were obtained from all the women at their first visit, using a questionnaire to retrieve details of their clinical and obstetric history. Also, anthropometric and lifestyle factors were collected and analysed, such as physical activity, using the short version of IPAQ (Hernandez-Cordero et al., 2008; Bertolotto et al., 2010; Harizopoulou et al., 2010). The cut-off points for the above variables are presented in Chapter 2.

During the first visit, random blood samples were collected to measure blood glucose, insulin, HbA1c, and lipid profile. During mid-pregnancy (at 24-28 weeks of gestation), subjects were asked to fast for at least 10 hours, and GDM screening was performed. A fasting blood sample was then withdrawn and each participant was subsequently made to drink 75 gm of glucose within five minutes. Blood samples were collected after 60 and 120 minutes, for the assessment of glucose and insulin levels. IADPSG guidelines were also applied to diagnose GDM as described in chapter 2. HOMA-IR and HOMA- $\beta$  were also calculated using HOMA-IR and HOMA- $\beta$  Matthews equations, respectively (Matthews et al., 1985; Wallace et al., 2004).

An assessment of other biochemical parameters performed at the first visit was also repeated, including HbA1c and lipid profile. Finally, metabolic syndrome was classified according to the criteria proposed by the US NHLBI/AHA, with adaptations appropriate for the present study population (Grundy et al., 2005). Metabolic syndrome is defined in Chapter 2.

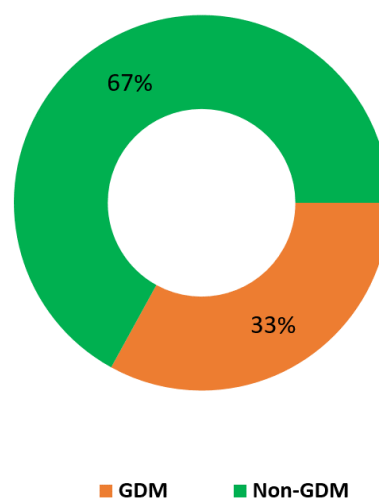
### **5.2.3 Data Analysis**

Data were analysed using SPSS version 21.0 statistical software (SPSS, Chicago, IL, USA). Pearson's chi-square test was applied to observe the association between categorical variables (GDM vs. non-GDM). To determine GDM and non-GDM differences, an independent sample t-test and Mann-Whitney U Test were implemented for normally and non-normally distributed variables, respectively. ANCOVA was applied to compare groups using age and BMI as covariates. To compare non-normally distributed variables, categorised into more than two groups, an independent sample Kruskal Wallis Test was conducted. Pearson's and Spearman's rank correlation coefficients were also determined to assess the linear relationship between quantitative variables for normally and non-normally distributed variables, respectively, with a p-value of  $< 0.05$  indicating statistical significance.

## 5.3 Results

### 5.3.1 Prevalence of GDM

The present study included 297 consecutive pregnant women, 33% of whom (98/279) were diagnosed with GDM, based on IADPSG (**Figure 5.1**). According to their fasting blood glucose, 65.7% (195/297) of the sample were diagnosed with GDM, and 34.3% (101.8/297) were diagnosed using a 1-hour or 2-hour glucose tolerance test.

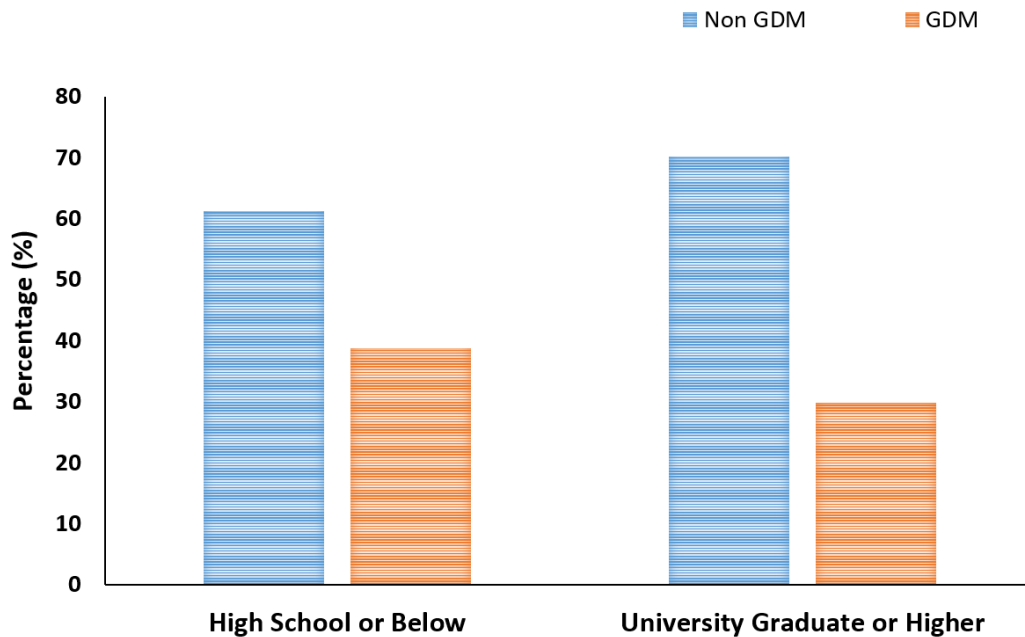


**Figure 5.1** The prevalence of GDM according to IADPSG. The values show the percentage of women with, and without, GDM by mid-pregnancy.

### 5.3.2 General Characteristics of Subjects with and without GDM

#### 5.3.2.1 Socio-economic Status in Relation to GDM

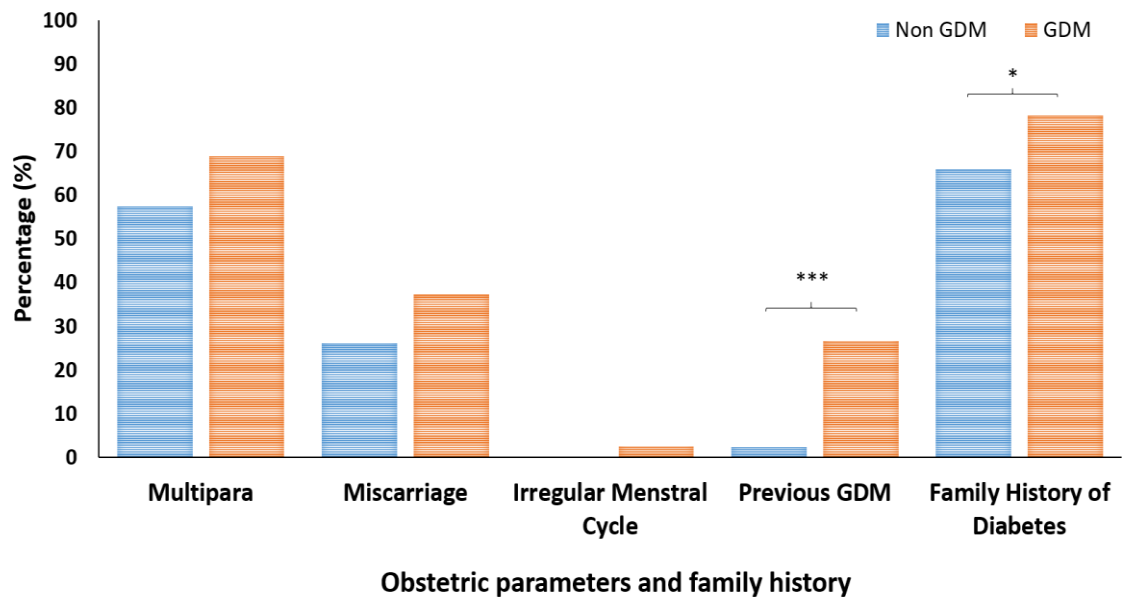
There were no significant socio-economic differences observed between women with and without GDM, in terms of their education, income status, employment, or place of residence. A lower proportion of the women were university graduates in the GDM group, compared to the non-GDM group, but this was not statistically significant (29.8% vs.70.2%,  $p = 0.107$ ) (**Figure 5.2**).



**Figure 5.2** Educational status of women, in relation to GDM status. The values show the percentage of women with university degrees among the GDM and non-GDM subjects. P-values were obtained using Pearson's chi-square test.

### 5.3.2.2 Obstetric and Family History in Relation to GDM

In the GDM group, the women were significantly more likely to have experienced GDM in the past, and have a family history of diabetes, compared with the women who were not currently suffering from GDM ( $p < 0.001$  and  $p = 0.045$ , respectively). Multiparity and irregular menstrual cycle were also more common among the women suffering from GDM, compared to their counterparts without GDM, but this was not statistically significant ( $p = 0.067$  and  $p = 0.097$ , respectively). Data on obstetric parameters and family history are presented in **Figure 5.3**.



**Figure 5.3** Obstetric parameters and patient history, in relation to GDM status. The values show the percentage of women with obstetric risk factors, and a family history of diabetes in the GDM and non-GDM groups. P-values: \* denotes  $p < 0.05$  and \*\*\* denotes  $p < 0.001$  using Pearson's chi-square test.

### 5.3.2.3 Anthropometric Characteristics in Relation to GDM

Pre-pregnancy, early, and mid-pregnancy BMIs were significantly higher in the GDM group, compared to their counterparts without GDM, but this significance disappeared after adjusting for age. Additionally, waist circumference in early and mid-pregnancy was significantly higher amongst the GDM subjects, compared with the non-GDM subjects, but the difference was insignificant after adjusting for age and BMI. No significant differences were observed in WHR, body fat, or  $\Delta$  body fat percentage during either visit. The anthropometric characteristics of both the GDM and non-GDM groups are shown in **Table 5.1**. Furthermore, maternal fasting glucose showed a positive correlation with pre-pregnancy BMI ( $r = 0.18$ ,  $p = 0.002$ ), and BMI in early ( $r = 0.18$ ,  $p = 0.002$ ), and mid-pregnancy ( $r = 0.16$ ,  $p = 0.007$ ), along with gestational age ( $r = 0.16$ ,  $p = 0.006$ ).

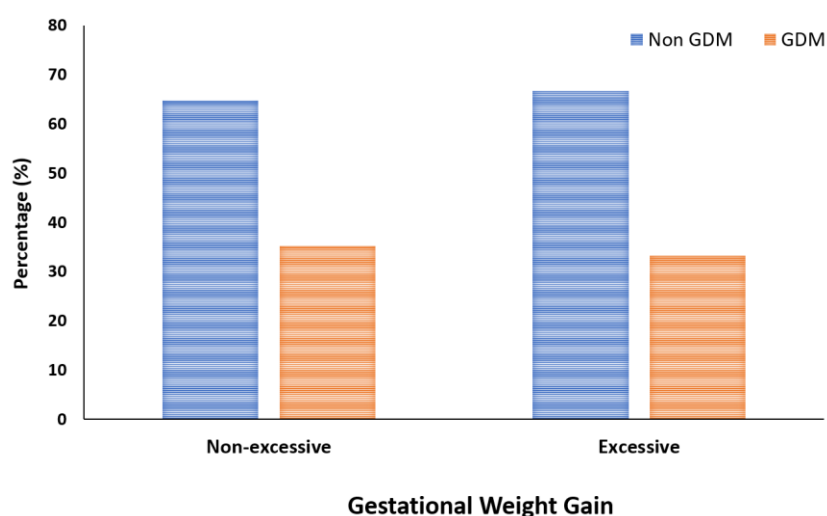
**Table 5.1 Anthropometric characteristics for both visits, in relation to GDM status**

Parameters	Non GDM (N = 198)	GDM (N = 99)	P-values	P-values *
<b>Booking Visit</b>				
Age (years)	28.5 ± 5.3	29.6 ± 5.4	0.098	---
Gestational age (weeks)	12.1 ± 2.9	12.4 ± 3.3	0.391	0.399
Pre-pregnancy BMI (kg/m <sup>2</sup> )	26.1 ± 6.0	28.3 ± 5.7	0.003	0.111
BMI (kg/m <sup>2</sup> ) at visit 1	27.3 ± 6.4	29.2 ± 5.5	0.010	---
Waist circumference (cm)	91.0 ± 12.9	94.3 ± 12.9	0.040	0.402
Hip circumference (cm)	107.1 ± 12.2	109.4 ± 10.6	0.108	0.738
Waist-hip ratio (WHR)	0.9 ± 0.1	0.9 ± 0.1	0.198	0.253
Systolic BP (mmHg)	114.6 ± 13.8	113.1 ± 12.8	0.359	0.115
Diastolic BP (mmHg)	68.0 ± 9.9	66.9 ± 9.5	0.359	0.196
Body fat (%)	33.5 ± 6.4	34.3 ± 4.6	0.292	0.906
<b>OGTT Visit</b>				
Gestational age (weeks)	26.4 ± 3.0	26.1 ± 3.6	0.463	0.476
BMI (kg/m <sup>2</sup> ) at visit 2	29.5 ± 6.4	31.1 ± 5.2	0.035	0.228
Gestational weight gain (GWG) (kg)	7.5 ± 4.2	6.5 ± 4.3	0.054	0.211
Waist circumference (cm)	103.8 ± 13.0	107.1 ± 12.5	0.041	0.358
Hip circumference (cm)	112.7 ± 11.5	114.9 ± 10.7	0.126	0.263
Waist-hip ratio (WHR)	0.9 ± 0.1	0.9 ± 0.1	0.094	0.053
Systolic BP (mmHg)	111.2 ± 12.1	113.4 ± 13.0	0.163	0.718
Diastolic BP (mmHg)	67.2 ± 10.2	69.3 ± 11.2	0.106	0.249
Body fat (%)	37.1 ± 3.5	37.3 ± 2.9	0.711	0.686
Δ Body fat (%)	10.1 ± 28.9	10.3 ± 14.9	0.952	0.437

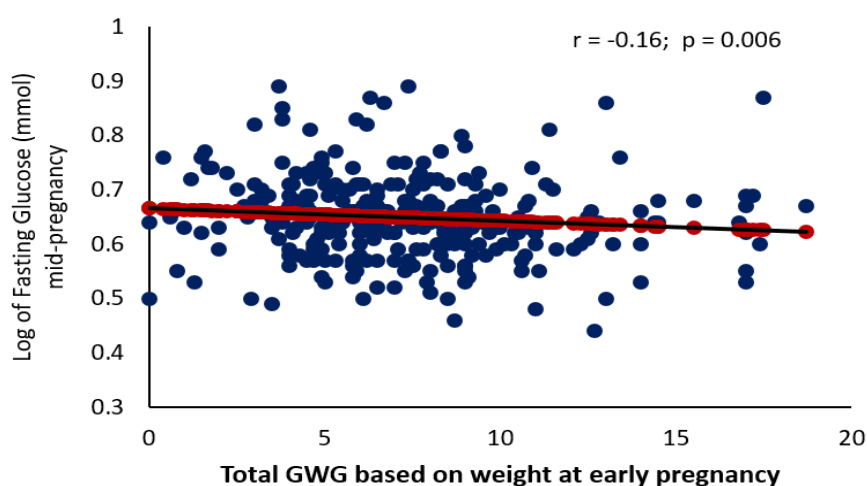
**Note:** Data are presented as the mean and standard deviation for normal variables. The p-value for mean differences was obtained using an independent sample t-test for normally distributed variables. \* indicates p-values adjusted for age and BMI, at the first visit.



For GWG, either in total kilograms (**Table 5.1**) or, according to the IOM (excessive/non-excessive), no difference was found between the groups (**Figure 5.4**). Interestingly, a negative correlation was detected between total GWG and fasting glucose ( $r = -0.16$ ,  $p = 0.006$ ) (**Figure 5.5**). What is more, total GWG was found to be significantly lower among women who had been obese pre-pregnancy, in comparison to their underweight, normal, and overweight counterparts ( $p < 0.001$ ) (**Appendix VI**).



**Figure 5.4** GWG parameters, based on IOM mid-pregnancy, in relation to GDM status. The values show the percentage of women with excessive and non-excessive weight gain by mid-pregnancy, in both the GDM and non-GDM groups. Pearson's chi-square test was used.



**Figure 5.5** Correlation between log of fasting glucose (mmol/L) and GWG, between early and mid-pregnancy.

#### **5.3.2.4 Biochemical Characteristics in Relation to GDM**

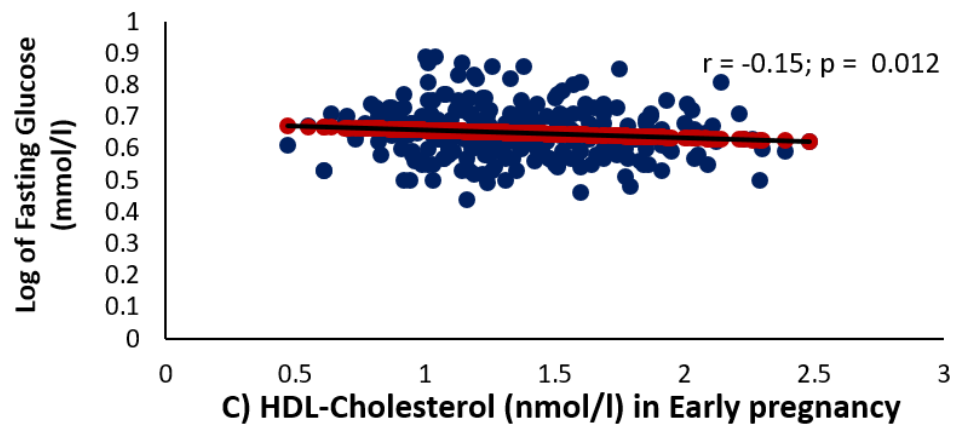
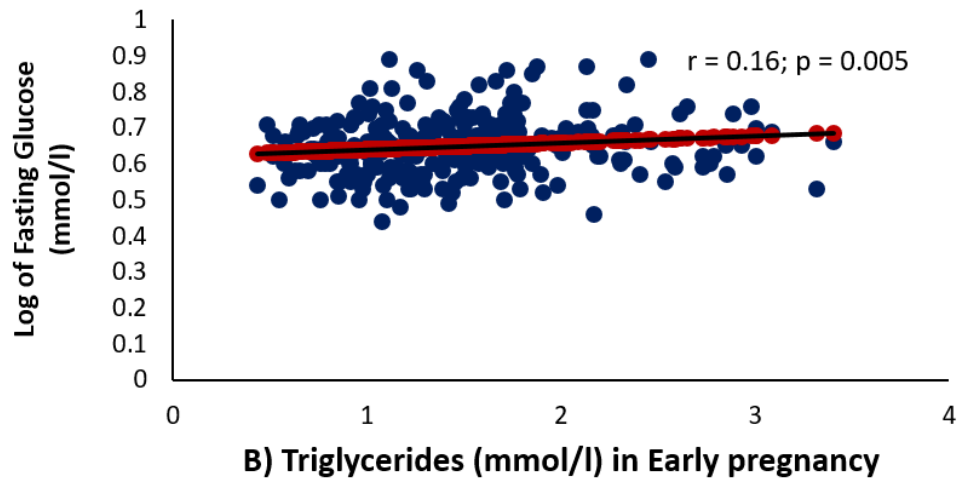
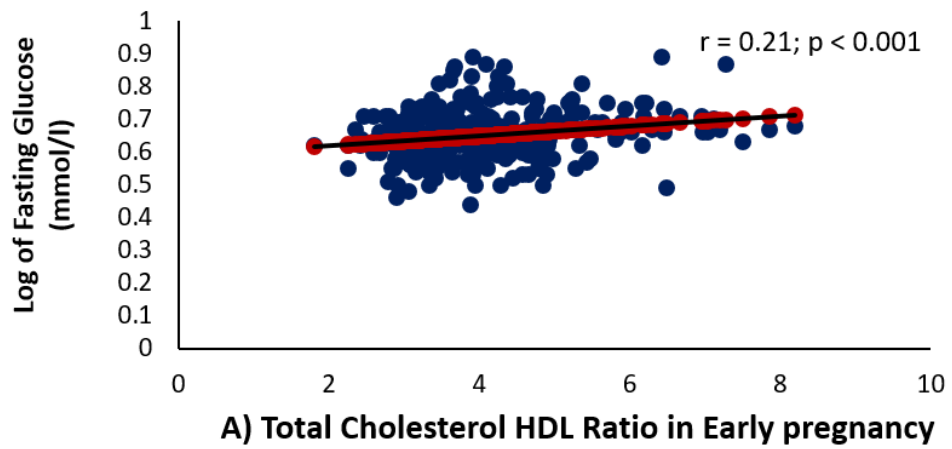
The biochemical parameters in both early and mid-pregnancy are shown in **Table 5.2**. Maternal fasting glucose in the OGTT exhibited a positive correlation with random glucose ( $r = 0.15$ ,  $p = 0.012$ ), random insulin ( $r = 0.13$ ,  $p = 0.028$ ), 1- and 2-hour glucose tolerance ( $r = 0.25$ ,  $p < 0.001$  and  $r = 0.22$ ,  $p < 0.001$ ), HbA1c at both visits ( $r = 0.24$ ,  $p < 0.001$  and  $r = 0.29$ ,  $p < 0.001$ ), fasting insulin ( $r = 0.38$ ,  $p < 0.001$ ), 2-hour insulin ( $r = 0.14$ ,  $p = 0.056$ ), and HOMA-IR ( $r = 0.54$ ,  $p < 0.001$ ). Furthermore, maternal fasting glucose showed a negative correlation with HOMA- $\beta$  ( $r = -0.37$ ,  $p < 0.001$ ).

The lipid profile at both visits, except for TG, failed to show any significant difference between the GDM groups. TG in mid-pregnancy was significantly higher among the GDM sufferers, compared to the non-GDM group, persisting even after adjusting for BMI and age. A positive correlation was observed between maternal fasting glucose and total cholesterol/HDL ratio in early ( $r = 0.21$ ,  $p < 0.001$ ) and mid-pregnancy ( $r = 0.19$ ,  $p < 0.001$ ), and TG in early ( $r = 0.16$ ,  $p = 0.005$ ) and mid-pregnancy ( $r = 0.19$ ,  $p = 0.001$ ). Also, maternal fasting glucose correlated negatively with HDL-cholesterol in early ( $r = -0.15$ ,  $p = 0.012$ ) and mid-pregnancy ( $r = -0.20$ ,  $p = 0.001$ ). These correlations presented in early (**Figure 5.6**) and mid-pregnancy (**Figure 5.7**).

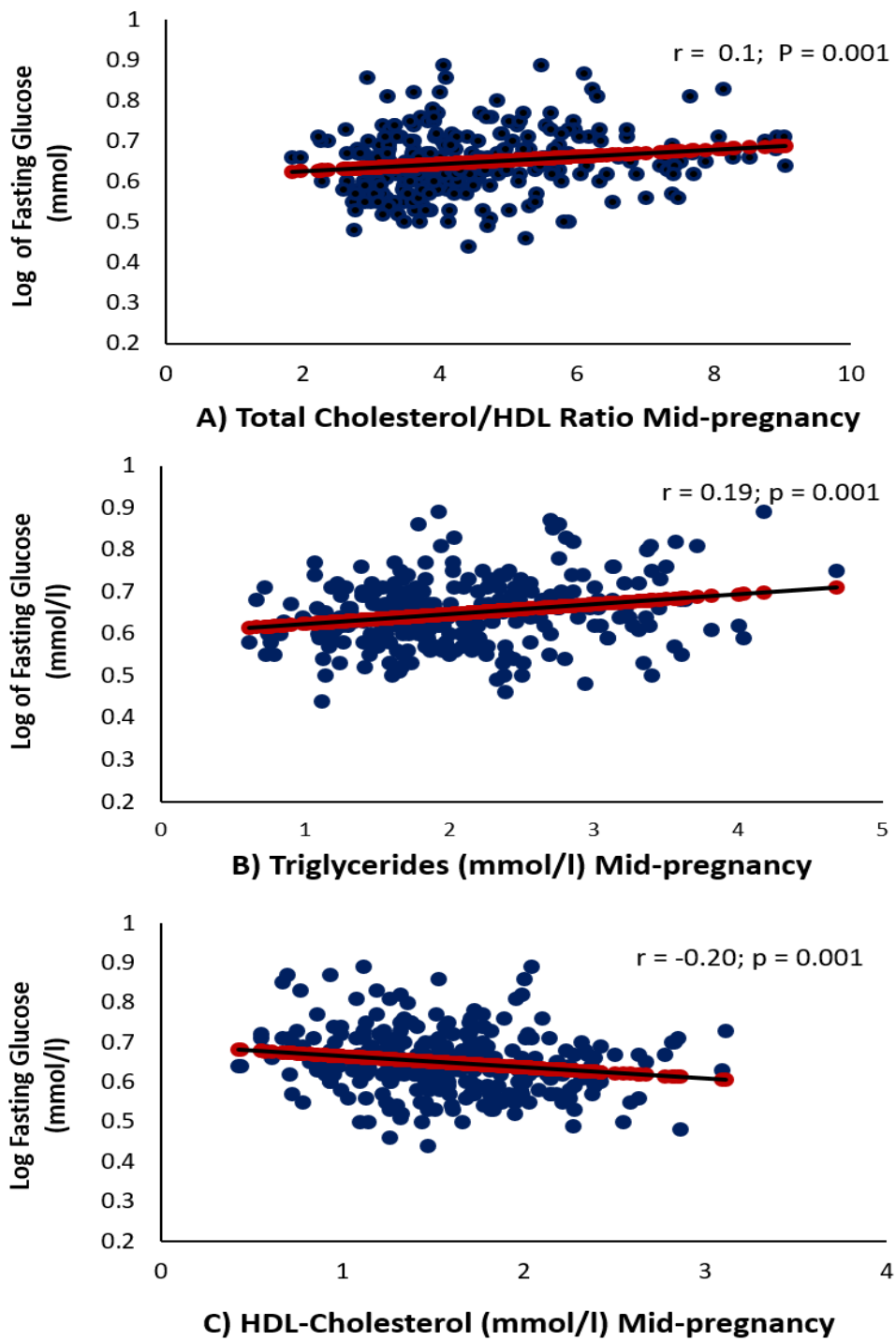
**Table 5.2 Biochemical characteristics in early and mid-pregnancy in relation to GDM status**

Parameters	Non-GDM (N = 198)	GDM (N = 99)	P-values	P-values*
<b>Booking Visit</b>				
<b>Glucose (mmol/L) #</b>	4.6 (4.3 - 5.2)	5.1 (4.6 - 5.8)	<b>&lt; 0.001</b>	<b>0.002</b>
Insulin (uU/mL) #	8.2 (4.2 - 18.3)	9.6 (6.0 - 20.6)	0.059	0.375
<b>HbA1c (%)</b>	5.0 ± 0.5	5.2 ± 0.5	<b>&lt; 0.001</b>	<b>0.003</b>
Total cholesterol (mmol/L)	5.2 ± 1.0	5.3 ± 1.0	0.336	0.930
HDL-cholesterol (mmol/L)	1.4 ± 0.4	1.3 ± 0.4	0.206	0.185
Cholesterol-HDL ratio	4.0 ± 1.0	4.2 ± 1.2	0.061	0.187
LDL-cholesterol (mmol/L)	3.1 ± 0.7	3.2 ± 0.8	0.449	0.920
Triglycerides (mmol/L)	1.4 ± 0.6	1.6 ± 0.6	0.071	0.253
<b>OGTT Visit</b>				
<b>Fasting glucose (mmol/L) #</b>	4.2 (3.8 - 4.5)	5.2 (4.7 - 5.7)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
<b>1-hr glucose (mmol/L) #</b>	6.8 (5.6 - 8.0)	9.5 (7.9 - 10.7)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
<b>2-hr glucose (mmol/L) #</b>	6.0 (5.1 - 7.0)	7.1 (5.9 - 9.5)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
<b>Fasting insulin (uU/mL) #</b>	6.2 (4.2 - 10.8)	10.5 (6.7 - 17.8)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
2-hr insulin (uU/mL) #	35.3 (24.1 - 53.9)	55.5 (32.7 - 76.8)	< 0.001	0.124
<b>HOMA-IR #</b>	1.2 (0.8 - 2.2)	2.4 (1.6 - 4.6)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
HOMA-β #	201.8 (127.5 - 387.4)	168.9 (74.8 - 270.3)	0.002	0.194
<b>HbA1c (%)</b>	4.8 ± 0.4	5.0 ± 0.5	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
Total cholesterol (mmol/L)	6.6 ± 1.3	6.7 ± 1.4	0.656	0.857
HDL-cholesterol (mmol/L)	1.6 ± 0.5	1.5 ± 0.5	0.154	0.080
Cholesterol-HDL ratio	4.5 ± 1.7	4.9 ± 2.0	0.057	0.075
LDL-cholesterol (mmol/L)	4.1 ± 1.1	4.1 ± 1.1	0.748	0.732
<b>Triglycerides (mmol/L)</b>	2.0 ± 0.7	2.3 ± 0.8	<b>0.001</b>	<b>0.005</b>

**Note:** Data presented as the mean and standard deviation for normally distributed variables, while the median, 1<sup>st</sup> and 3<sup>rd</sup> quartiles are presented for non-normally distributed variables. # indicates non-normally distributed variables; the p-value for mean differences was obtained using an independent sample t-test for normal variables, and a Mann-Whitney U Test for non-normally distributed variables; \* indicates p-values, adjusted for age and BMI at the 1<sup>st</sup> visit.



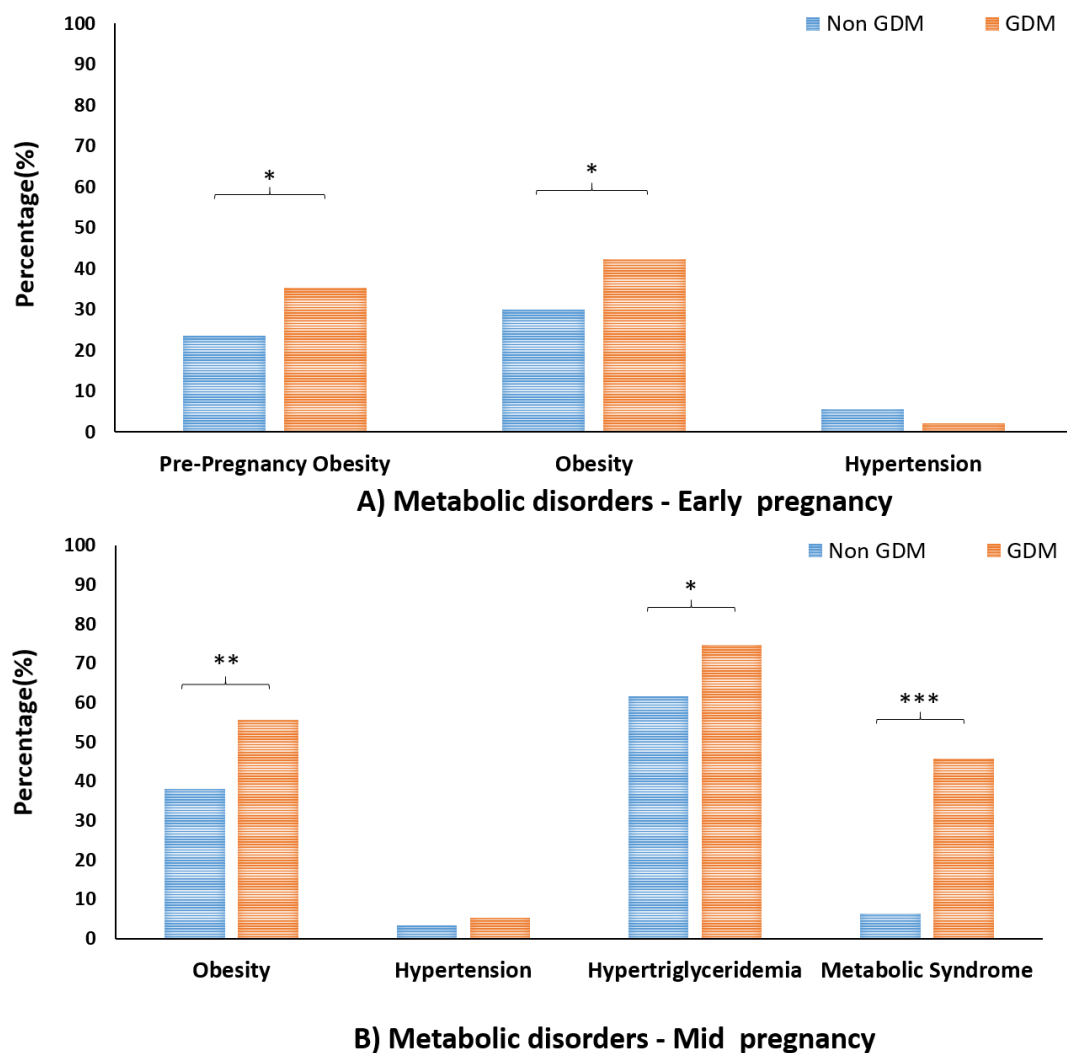
**Figure 5.6** Correlations between the log of fasting glucose, and (A) Total cholesterol-HDL ratio, (B) Triglycerides, and (C) HDL-cholesterol in early pregnancy.



**Figure 5.7** Correlations between the log of fasting glucose, and (A) Total cholesterol-HDL ratio, (B) Triglycerides, and (C) HDL-cholesterol mid-pregnancy.

### 5.3.2.5 Metabolic Disorders in Relation to GDM Status

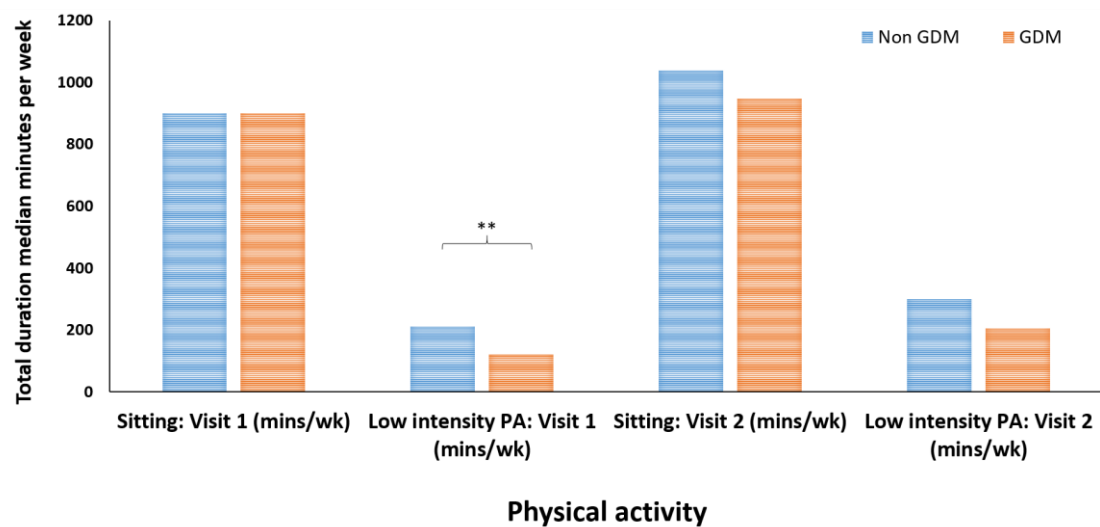
The prevalence of pre-pregnancy obesity, and obesity in early and mid-pregnancy, were higher among the subjects with GDM compared with those without GDM (**Figure 5.8**). At mid-pregnancy, the percentage of participants with hypertriglyceridemia or metabolic syndrome was also significantly higher among the subjects with GDM, in comparison with their non-GDM counterparts ( $p = 0.024$  and  $p < 0.001$ , respectively). This will be discussed further in Chapter 6.



**Figure 5.8** Presence of metabolic disorders in (A) Early, and (B) Mid-pregnancy among GDM and non-GDM subjects. The values show the percentage of women with different metabolic disorders in early and mid-pregnancy, in both the GDM and non-GDM groups. P-values: \* denotes  $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ , using Pearson's chi-square test.

### 5.3.2.6 Physical Activity in Relation to GDM Status

In early pregnancy, women without GDM undertook significantly higher low-intensity physical activity/walking (in minutes/week), compared with the GDM subjects, even after adjustment for age and BMI ( $p = 0.007$ ). However, the latter variable was not statistically significant by mid-pregnancy ( $p = 0.163$ ) (**Figure 5.9**).



**Figure 5.9** Physical activity (PA) at both visits, in relation to GDM status. The values show the median sedentary and physical activity in minutes per week among both the GDM and non-GDM subjects, after adjusting for age and BMI. \*\* denotes  $p < 0.01$ , using the Mann-Whitney U Test.

## 5.4 Discussion

This study is one of the few studies conducted in Saudi Arabia that assesses the prevalence of GDM using IADPSG, along with the associated risk factors in both early and mid-pregnancy. In this present chapter, it was determined that 33% of the subjects had developed GDM by mid-pregnancy. Moreover, in early pregnancy, higher random blood glucose and HbA1c levels were associated with GDM in mid-pregnancy, whereas hypertriglyceridemia and metabolic syndrome in mid-pregnancy were higher in the GDM group. A maternal lifestyle factor, such as physical activity, was associated with conferring protection against GDM. Many of these risk factors are adjustable, so timely identification of the determinants, and subsequent intervention could prevent the development of GDM, and any associated morbidity.

The prevalence of GDM in Saudi Arabia has been steadily increasing, from 12.5% in 2000, to 18.7% in 2013, and then 24% in 2015, according to the ADA and WHO 2013 criteria (Ardawi et al., 2000; Wahabi et al., 2014; Wahabi et al., 2016). A prevalence of 33% GDM was found in the current study, according to the IADPSG criteria. This appears to be high, thus corresponding to the rising trend towards GDM in Saudi Arabia. The IADPSG criteria may even have contributed to the observed prevalence of GDM in this present study, as this is 2-3 times higher than the previously reported prevalence of GDM in the respective context, when the ADA criteria were used (Al-Rowailly & Abolfotouh, 2010; Wahabi et al., 2014). The reason for the high prevalence when using the IADPSG criteria may be due to the inclusion of pregnant women with hyperglycaemia in the GDM group (Han et al., 2012; IDF, 2013). In support of the findings from the present study, the application of the IADPSG criteria has previously determined 39.6% and 36.6% prevalence rates of GDM in Saudi Arabia, and 37.7% in the UAE (Agarwal et al., 2010; Al-Rubeaan et al., 2014; Alfadhli et al., 2015).

The difference between the prevalence rates in the present study, and a study conducted by Al-Rubeaan et al. (2014) is that the latter used fasting glucose levels alone to screen for GDM. This was due to convenience, as it was a home-based study. Alfadhli et al. (2015) reported a higher incidence of GDM in Western Saudi Arabia, but the present



study was conducted in the central region, so regional variation could account for some of the differences between the studies. The application of IADPSG to assess GDM in other international studies has reported prevalence rates amounting to 30.5% in Norway (Jenum et al., 2012), 29.9% in Iran (Shahbazian et al., 2016), and 27% in India (Nayak et al., 2013). Prevalence rates as low as 9.3% have been reported in China (Leng et al., 2015), 12.4 % in Ireland (O'Sullivan et al., 2011), 13% in Australia (Moses et al., 2011), and 17.8% in Canada (Ryan, 2011). It is believed that the prevalence of GDM is influenced by ethnic variations within a population, as well as genetic, demographic, socio-cultural, and socio-economic factors (Yuen & Wong, 2015).

A family history of diabetes, prior history of GDM, and multiparity were well-known factors associated with GDM, identified in this and previous studies (Savvidou et al., 2010; Teh et al., 2011; Khan et al., 2013; Alfadhli et al., 2015; Erem et al., 2015; Chitme et al., 2016; Lin et al., 2016; Shahbazian et al., 2016). It is worth noting that the percentage of women with a family history of diabetes in the GDM group, in the current study, amounted to 78.3%, which is similar to the previously reported 68.2% of pregnant Saudi women (Alfadhli et al., 2015). The higher prevalence of a family history of diabetes in both Saudi cohorts paralleled the high prevalence of T2DM in Saudi Arabia (Al-Nozha et al., 2004; Al-Daghri et al., 2010; Al-Rubeaan et al., 2014). Irregular menstrual cycle in this cohort showed a higher trend in GDM subjects, compared to non-GDM subjects ( $p = 0.097$ ). Other studies have previously revealed that an irregular menstrual cycle is significantly associated with GDM (Haver et al., 2003; Bhat et al., 2010).

It is well recognised that pre-pregnancy obesity, or obesity at different gestational stages is associated with GDM, and this was supported by the current study, as well as previous studies in different countries, including Saudi Arabia (Al-Rowaily & Abolfotouh, 2010; El-Gilany & Hammad, 2010; Bener et al., 2013; Al-Rubeaan et al., 2014; Park et al., 2014; Arnold et al., 2015; Erem et al., 2015; Mao et al., 2015; Lin et al., 2016; Shahbazian et al., 2016). However, the present study revealed no difference in fat mass markers between the GDM and non-GDM groups, in either early or mid-

pregnancy. This may be attributed to foetal growth and fluid retention in GDM mothers, which may have affected the readings of fat mass in this study.

In the present study, a negative association between fasting glucose and GWG was detected. This inverse correlation between pregnancy-related weight gain and GDM has previously been reported (Nohr et al., 2008; Heude et al., 2012; Li et al., 2015; Hung & Hsieh, 2016). This could be explained by women with a higher BMI gaining less weight, because they are more cautious about it. However, even if these women gain less weight in pregnancy, their hepatic gluconeogenesis may still be bad, because of their pre-pregnancy obesity. In the current study, irrespective of GDM status, total GWG in kilograms or GWG according to the IOM (2009), the GDM group also demonstrated lower weight gain from early to mid-pregnancy, but this did not reach a level of significance. Other earlier studies have also failed to indicate a difference (Saldana et al., 2006; Herring et al., 2009; Lacroix et al., 2014; Park et al., 2014; Ruifrok et al., 2014). On the contrary, there is evidence to suggest that the increased risk of developing GDM during pregnancy is related to excessive weight gain before GDM diagnosis (Brunner et al., 2015). Inconsistencies in studies regarding the association of GWG and GDM could be due to differences between the cut-off points, continuous and other categorical measurements and the time intervals for assessing GWG (Dode & Santos, 2009).

In addition to the above, low-intensity physical activity for a minimum of 30 minutes/day during early pregnancy was found to be linked with GDM in this instance. Similar observations have also been reported in numerous studies documenting that light-to-moderate intensity physical activity during early pregnancy is linked with a lower risk of GDM (Oken et al., 2006; Morkrid et al., 2014; Nasiri-Amiri et al., 2016). An assessment of physical activity using the IPAQ-Greek version revealed that inactivity among pregnant women during the early stages of pregnancy poses a significant risk of GDM, compared to women involved in physical activities (Harizopoulou et al., 2010). An example of this could be regular walk during early pregnancy, which has been shown to have an inverse relationship with the risk of GDM, whereas occupational and physical activity in the home has no impact (Aune et al.,

2016). However, this current study, along with others, failed to find an association between mid-pregnancy physical activity and GDM (Parildar et al., 2013; Chasan-Taber et al., 2014), which points to the relevance of physical activity during early pregnancy, but not during the later stages, when GDM may have already occurred.

Physical activity has been linked to a lowered risk of excessive GWG, insulin resistance, and T2DM (Helmrich et al., 1991; Manson et al., 1991; Wang et al., 2002). It has also long been known for its role in improving glucose homeostasis by enhancing insulin sensitivity, and this is most likely due to increasing fat-free mass (Shulman et al., 1990; Annuzzi et al., 1991; Devlin, 1992). Moreover, physical activity has been implicated in the prevention or delay of T2DM onset among non-pregnant individuals (Boule et al., 2001; Jeon et al., 2007). It is, therefore, quite likely that physical activity has the potential to prevent the development of GDM, and its related adverse health outcomes (Morkrid et al., 2014).

In the present study, fasting glucose by mid-pregnancy was diagnostic for GDM in 65.7% of cases. Therefore, the assessment of fasting glucose appears to be a useful option as an initial screening test to diagnose GDM (Agarwal et al., 2010). Moreover, the assessment of 1- and 2-hour glucose concentrations during the OGTT was also useful for GDM diagnosis, as these tests were diagnostic in over one-third of the GDM cases here. The above findings differed from those derived from another Saudi study, where 48% GDM cases were diagnosed by fasting glucose, and 52% were diagnosed using 1- and 2-hour OGTT glucose levels (Alfadhli et al., 2015).

Random plasma glucose, in early pregnancy, has been proposed as a useful screening test for GDM, identifying women at low and high risk of GDM (Jowett et al., 1987; Meek et al., 2016). Meek et al. (2016) suggest that performing a random blood glucose test, taking into account age and BMI, during the early stages of pregnancy, can improve sensitivity, but is of low specificity for GDM diagnosis (Meek et al., 2016). The results of one systematic review showed that random blood glucose and OGTT in the first trimester were good indicators of GDM (Van Leeuwen et al., 2011), and some older reports showed that GDM could be positively diagnosed in early pregnancy

(Super et al., 1991; Bartha et al., 2000; Bartha et al., 2003). However, the validity of applying the WHO 2013 criteria before 24–28 weeks of gestation has been questioned (Zhu et al., 2013).

HbA1c percentages during both visits were within normal limits, however, the mean HbA1c level in the GDM group was significantly higher than the non-GDM group, even after adjustment for age and BMI. A relatively low percentage of HbA1c (5.2% vs. 5%) was detected in the present study, during early pregnancy in the GDM and non GDM group, respectively. Yet, HbA1c value of 5.4% among women with GDM has also been reported (Kwon et al., 2015). A cut-off value of  $\text{HbA1c} \geq 6.5\%$  is considered to be diagnostic for diabetes in pregnancy (Metzger et al., 2010; WHO, 2013; ADA, 2014), but this is based on data for non-pregnant subjects. Moreover, first trimester  $\text{HbA1c} \geq 6.1\%$  has been shown to exhibit a strong association with the future development of GDM (Anaka et al., 2014). The optimal HbA1c threshold in pregnancy is likely to be lower, as HbA1c levels fall in the first trimester (Nielsen et al., 2004; Mosca et al., 2006), which could be due to the shorter lifespan of red blood cells during pregnancy (Lind & Cheyne, 1979; Lurie & Danon, 1992). For the same reason, a cut-off of  $\text{HbA1c} \geq 5.9\%$ , at less than 20 weeks of gestation, has been recommended for the diagnosis of GDM (Hughes et al., 2014). The association of HbA1c with GDM in mid-pregnancy has already been documented (Wang et al., 2012; Kwon et al., 2015), whereas no association of HbA1c during the third trimester of pregnancy has been established for GDM (El Lithy et al., 2014).

Furthermore, the cardio-metabolic profile in this study was higher in subjects with GDM, compared to the GDM group, including fasting insulin, HOMA-IR, and TG. Fasting glucose positively correlated with the total cholesterol/HDL ratio, and negatively correlated with HOMA- $\beta$  and HDL-cholesterol, findings which support those of previous studies (Wang et al., 2012; Zhou et al., 2012; Pleskačová et al., 2015; Ryckman et al., 2015; Chitme et al., 2016).

Little is currently known about the relationship between metabolic changes during early pregnancy and GDM development (Chatzi et al., 2009; Lei et al., 2016). In this study,

it was difficult to assess metabolic syndrome during the first visit because of the non-fasting collection of blood samples in early pregnancy. In mid-pregnancy, however, the percentage of metabolic syndrome was significantly higher in the GDM group, compared to the non-GDM group. This relationship will be discussed further in the next chapter.

In conclusion, the prevalence of GDM was found to be high among the subjects in this study, namely pregnant Saudi women. It is crucial to identify the factors predisposing pregnant women to GDM, in order to ensure the timely detection of predisposition, and subsequently intervene to avoid the development of GDM, and its associated foetal and maternal morbidity.

## **Chapter 6**

### **Impact of Vitamin D Status on Gestational Diabetes Mellitus and Metabolic Risk Among Saudi Women**

## 6.1 Introduction

GDM is associated with insulin dysregulation, metabolic syndrome and multiple adverse maternal and neonatal complications (Kjos & Buchanan, 1999; Reece, 2010; Vilmi-Kerälä et al., 2015). Metabolic syndrome comprises a combination of glucose intolerance, abdominal obesity, dyslipidaemia, and HTN (Eckel et al., 2005). When GDM is coupled with a metabolic syndrome phenotype, it can also increase the future risk of cardiovascular disease and constitute a significant burden on healthcare resources (Alberti et al., 2005; Sullivan et al., 2012). Therefore, as GDM is a modifiable disorder, a better understanding of the pathogenesis of metabolic syndrome is critical for avoiding associated morbidity, through timely therapeutic intervention.

Several non-traditional risk factors have been implicated in the development of GDM. As such, vitamin D is considered to be an important predisposing factor influencing the development of GDM, due to its association with insulin resistance, inflammation, and dyslipidaemia (Norman et al., 1980; Boucher et al., 1995). Particularly as low serum concentrations of vitamin D have been shown to be associated with a higher risk of developing T2DM (Gagnon et al., 2011; Khan et al., 2013) and GDM (Zhang et al., 2008).

Currently, the majority of studies investigating the association between vitamin D deficiency and GDM have been performed during the second or third trimester of pregnancy (Farrant et al., 2008; McLeod et al., 2012; Wang et al., 2012; Whitelaw et al., 2014). Although, some studies have also investigated the first trimester of pregnancy before the development of GDM (Baker et al., 2012; Lacroix et al., 2014; Park et al., 2014; Jain et al., 2015). There is, however, growing evidence through systematic reviews that vitamin D deficiency is a substantial risk factor for the occurrence of GDM (Christesen et al., 2012; Poel et al., 2012; Aghajafari et al., 2013; Wei et al., 2013; Burris & Camargo, 2014; Zhang et al., 2015; Lu et al., 2016). Although most of the findings linking vitamin D deficiency with GDM are cross-sectional or case-control studies, there is a need to conduct targeted prospective longitudinal studies.

Beyond association studies, a number of mechanisms have been suggested connecting vitamin D deficiency with the development of GDM. The first of these mechanisms is considered to act through the direct binding of vitamin D to its receptor on a  $\beta$ -cell, which appears to enhance insulin secretion (Johnson et al., 1994). Similarly, the expression of 1- $\alpha$ -hydroxylase in a  $\beta$ -cell has been shown to activate 25(OH)D (Bland et al., 2004). In addition, vitamin D-dependent changes in calcium flux have been shown to trigger insulin secretion from  $\beta$ -cells in the pancreas (Eliades & Pittas, 2009; Pittas & Dawson-Hughes, 2010). The second mechanism, possibly involved in increasing the risk of GDM, also acts through vitamin D, but by enhancing the expression of insulin receptors and insulin responsiveness, thus promoting insulin sensitivity (Vaidya & Williams, 2012). Beyond the risk of GDM, vitamin D deficiency has been associated with systemic inflammation, leading to increased insulin resistance and  $\beta$ -cell apoptosis (Alvarez & Ashraf, 2009; Eliades & Pittas, 2009). Collectively, the current evidence suggests that vitamin D deficiency predisposes the development of hyperglycaemia. Moreover, it has been found that the expression and production of CYP24A1 is increased in placental tissue among subjects with GDM, compared with normal placental tissue, and serum vitamin D levels have been shown to negatively correlate with the expression of CYP24A1 in the placenta (Cho et al., 2013).

It is quite conceivable that factors associated with 25(OH)D deficiency during gestation may also lead to the development of GDM. Insulin resistance is frequently observed among obese women, which could possibly be related to the increased prevalence of vitamin D deficiency in this segment of the population, particularly during pregnancy (Baptiste-Roberts et al., 2009) because sequestration of vitamin D could take place in adipose tissue, thus lowering systemic levels (Wortsman et al., 2000). However, exposure to the sun through activity can reduce this deficiency, insulin resistance, and the risk of GDM, which positively correlates to the amount of physical activity performed (Zhang et al., 2006). A higher prevalence of hypovitaminosis D among Asian, Hispanic, Middle Eastern, and African women has already been established, and is, possibly also leading to a higher prevalence of GDM amongst these ethnicities (Oleson et al., 2010; Lin et al., 2015).



Genetic variations associated with VDR polymorphisms, and the dysregulation of vitamin D metabolism, have been linked with susceptibility to different diseases (Levin et al., 2012). Additionally, factors such as increased oxidative stress, related to placental dysfunction, which alter the expression of VDRs and the binding protein, may adversely affect vitamin D homoeostasis, resulting in the development of GDM (Ma et al., 2012). As such, it is important to understand the influence of vitamin D deficiency during pregnancy within the Saudi population. Therefore, this chapter addresses the question of whether vitamin D deficiency is associated with GDM outcomes, and what other factors may influence this association. It also considers variables, such as glycaemic indices, metabolic syndrome, biomarkers, and lifestyle, which may influence vitamin D levels, as well as the consequences of low vitamin D levels.

## **6.2 Research Design and Methods**

A total of 297 healthy Saudi pregnant women, with a mean age of  $28.8 \pm 5.4$  years were included in the study. All participants were recruited during the first trimester (8–12 weeks) of their pregnancies, after fulfilling previously described inclusion criteria (**Chapter 2**). They were then followed up to the second trimester (24–28 weeks) of gestation. Anthropometric data, the interview questionnaire, and blood samples were collected from each participant during early and mid-pregnancy.

### **6.2.1 Biochemical Assessment**

Venous blood samples were collected from each participant at both visits. During the first trimester, non-fasting blood samples were obtained for the assessment of baseline parameters, including random glucose, insulin, HbA1c, lipid profile, 25(OH)D, calcium, phosphorus, albumin, creatinine and alkaline phosphatase. Mid-pregnancy fasting blood samples (>10 hours) were drawn from each participant to assess fasting blood glucose, fasting insulin, 1- and 2-hour OGTT, 2-hour insulin, HbA1c, complete lipid profile, HOMA-IR, HOMA- $\beta$ , 25(OH)D, calcium, phosphorus, albumin, creatinine, and alkaline phosphatase. The laboratory analysis was detailed in chapter 2.

### **6.2.2 Data Analysis**

Data were analysed using SPSS version 21.0 statistical software (SPSS, Chicago, IL, USA). Pearson's chi-square test was then utilised to observe any correlations between categorical variables. In order to determine group differences, an independent sample t-test and Mann-Whitney U Test were applied to normally and non-normally distributed variables, respectively. Adjustment for age and BMI as covariates between the groups was undertaken via ANCOVA, while ANOVA was conducted to compare normally distributed variables categorised into more than two groups (tertiles).

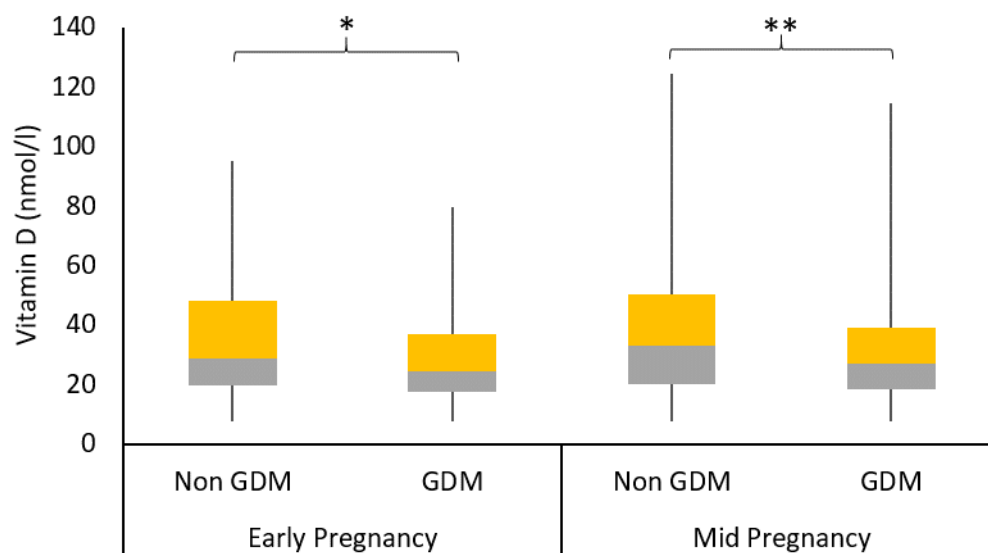
Pearson's and Spearman's rank correlation coefficients were used to determine any linear relationships between quantitative variables for normally and non-normally distributed variables, respectively. Logistic regression was also applied to identify the

risk factors associated with the categorical outcome variable. This method was selected based on the presence or absence of GDM as a dichotomous variable. Five models were subsequently generated to adjust for possible confounders, with Model 1: age and BMI at visit 1; Model 2: Model 1 + parity, education, employment, residence in North Riyadh, past GDM, family history (obesity, DM and GDM); Model 3: Model 2 + total GWG at visit 1 + changes in fat percentage; Model 4: Model 3 + sun exposure at visit 1+ season at visit 1+ vitamin D intake at visit 1, multivitamin intake at visit 1, and Model 5: Model 4 + physical activity at visit 1. All odds ratios were reported (unadjusted, adjusted for models 1, 2, 3, 4 and 5). Finally, a p-value of  $< 0.05$  was considered statistically significant.

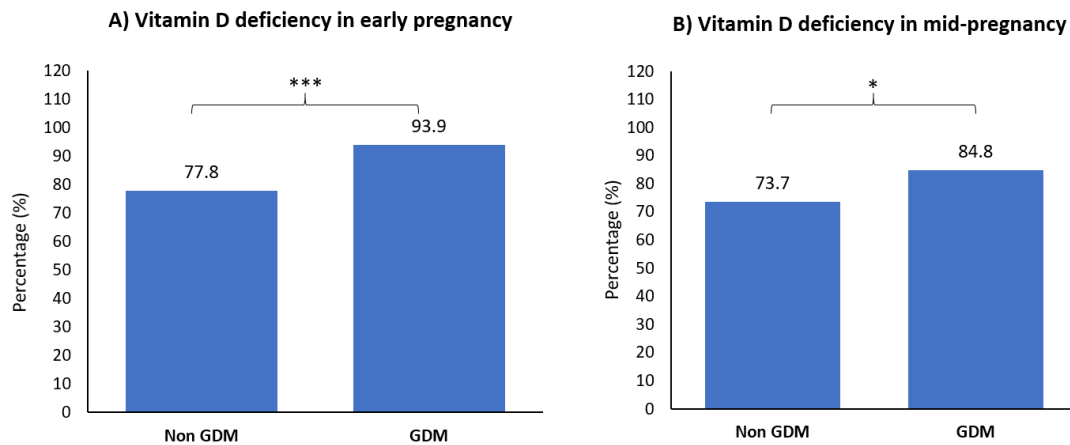
## 6.3 Results

### 6.3.1 Vitamin D Status and GDM

The median vitamin D level was lower in the GDM group, compared to the non- GDM group, in early [24.4 nmol/L (17.6-37.1) vs. 28.6 nmol/L (19.4-47.9),  $p = 0.033$ ] and mid-pregnancy [27.1 nmol/L (18.4-39.2) vs. 32.8 nmol/L (20.1-50.4),  $p = 0.014$ ], this remained significant after adjusting for age and BMI (**Figure 6.1**). Additionally, the prevalence of vitamin D deficiency in early and mid-pregnancy was higher among the GDM subjects than the non-GDM subjects (**Figure 6.2**).



**Figure 6.1** Vitamin D levels in early and mid-pregnancy in GDM and non-GDM subjects. The values show the median vitamin D level in both the GDM and non-GDM subjects, after adjusting for age and BMI. \* denotes  $p < 0.05$  and \*\* denotes  $p < 0.01$  using the Mann-Whitney U Test.

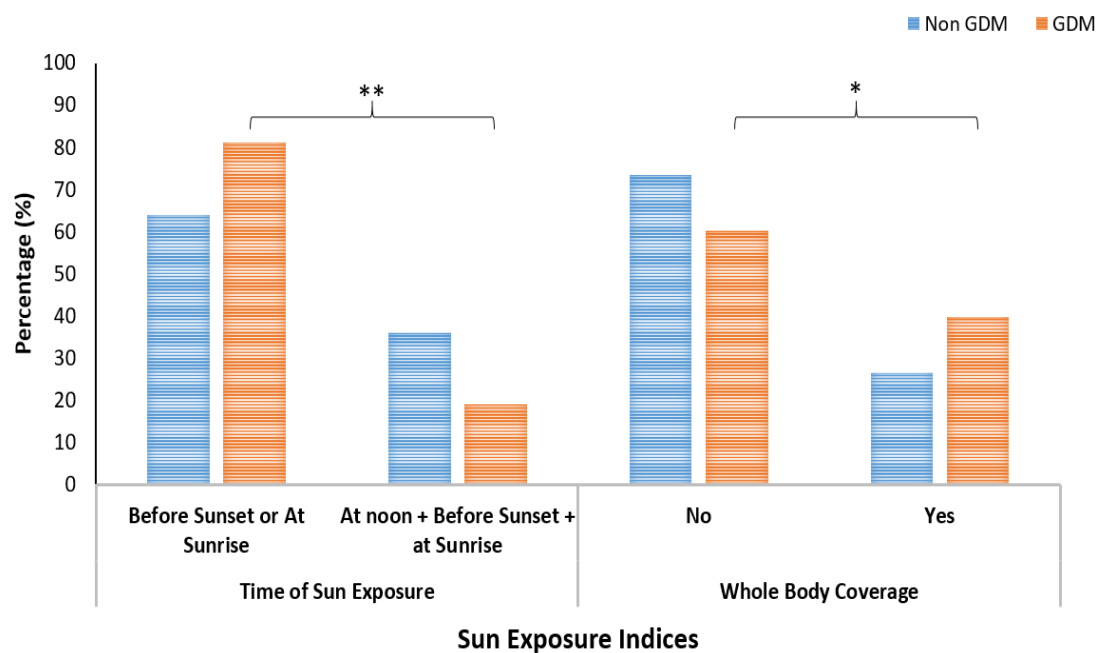


**Figure 6.2** The prevalence of vitamin D deficiency, in (A) Early and (B) Mid-pregnancy among GDM and non-GDM subjects. The values show the percentage of women with vitamin D deficiency in early and mid-pregnancy, in both the GDM and non-GDM groups. P-values: \* denotes  $p < 0.05$  and \*\*\* denotes  $p < 0.001$  using Pearson's chi-square test.

Interestingly, corrected calcium values were significantly lower among the subjects with GDM in early pregnancy, compared to their counterparts with no GDM ( $2.2 \text{ mmol/L} \pm 0.2$  vs.  $2.3 \text{ mmol/L} \pm 0.2$ ,  $p = 0.007$ ), and it remained significant, even after adjustment for age and BMI. In addition, alkaline phosphatase was significantly higher among the GDM women compared to their non-GDM counterparts, in early ( $10.6 \text{ mmol/L} \pm 3.4$  vs.  $9.3 \text{ mmol/L} \pm 2.8$ ,  $p = 0.001$ ), and mid-pregnancy ( $11.6 \text{ mmol/L} \pm 4.4$  vs.  $10.1 \text{ mmol/L} \pm 3.6$ ,  $p = 0.012$ ), which also remained significant after adjustment. Fasting glucose positively correlated with alkaline phosphatase at both visits ( $r = 0.20$ ,  $p = 0.002$ ;  $r = 0.24$ ,  $p < 0.001$ ). Finally, fasting glucose negatively correlated with phosphorus at mid-pregnancy ( $r = -0.19$ ,  $p = 0.003$ ).

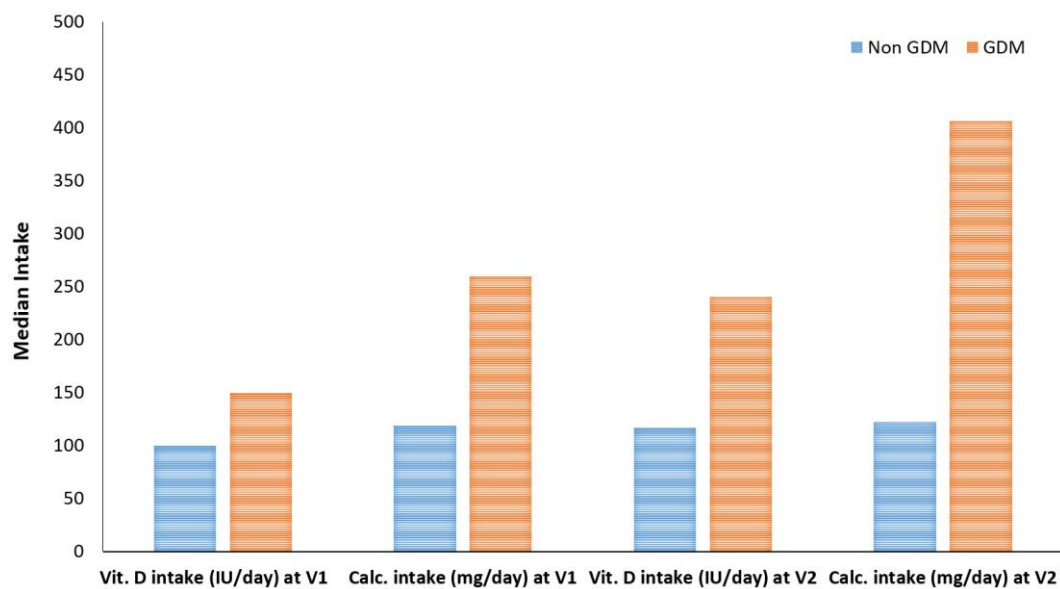
### 6.3.1.1 Influence of Lifestyle Related to Vitamin D Characteristics and GDM

There was no difference in sun exposure variables during early pregnancy, among the GDM subjects. At mid-pregnancy, the percentage of women covering their whole body was significantly higher in the GDM group than in the non-GDM group (39.7% vs. 26.6%,  $p = 0.040$ ). Similarly, the percentage of women exposed to the sun at noon was significantly higher among the non-GDM group, compared to the GDM group (36% vs. 18.9%,  $p = 0.004$ ) (**Figure 6.3**).

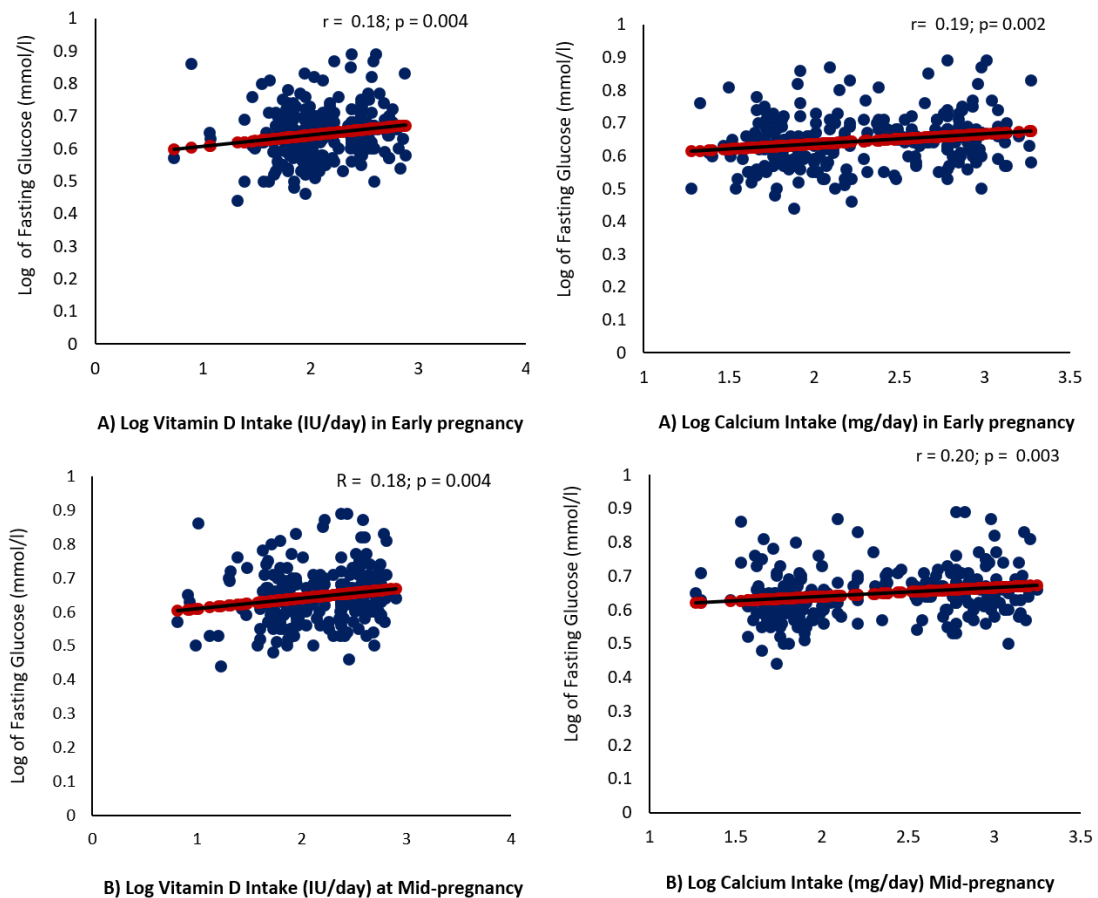


**Figure 6.3** Sun exposure at mid-pregnancy among the GDM and non-GDM subjects. The values show the percentage of women with different sun exposure indices in both the GDM and non-GDM groups. P-values: \* denotes  $p < 0.05$ , and \*\* denotes  $p < 0.01$ , using Pearson's chi-square test.

The present study revealed no differences between estimated vitamin D and calcium intake, irrespective of GDM status (**Figure 6.4**). Fasting glucose correlated positively with estimated vitamin D and calcium intake in early and mid-pregnancy (**Figure 6.5**). A higher proportion of the subjects without GDM were taking multivitamin supplements, compared to the subjects with GDM, at mid-pregnancy (53.9% vs. 41.9%), but this was not statistically significant ( $p = 0.095$ ). However, no differences were reported for multivitamin supplement intake in early pregnancy.



**Figure 6.4** Vitamin D and calcium intake at both visits, in relation to GDM status. The values show the median intake of vitamin D and calcium from food, among both the GDM and non-GDM subjects, after adjusting for age and BMI. ‘V1’ and ‘V2’ indicate 1st and 2nd visit, respectively. ‘Calc.’ indicates calcium. The Mann-Whitney U Test was applied.

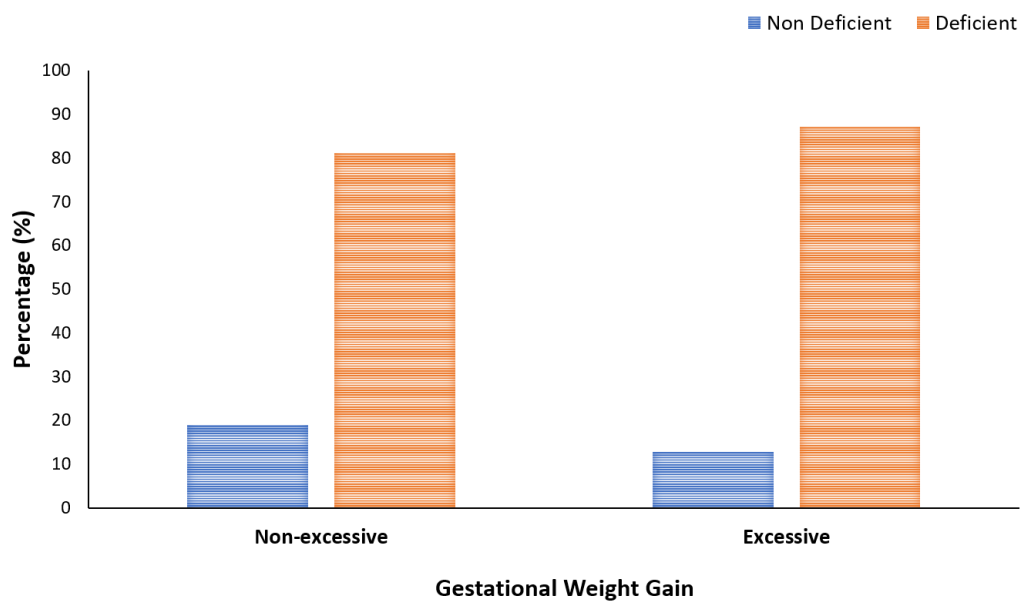


**Figure 6.5** Correlations between log of fasting glucose (mmol/L), vitamin D intake (IU/day), and calcium intake (mg/day) in (A) Early-pregnancy and (B) Mid-pregnancy.

### 6.3.2 Vitamin D and its Association with GDM Markers in Pregnant Women

No differences were observed prospectively between anthropometric data in mid-pregnancy and vitamin D-deficient and non-deficient women in early pregnancy. Although GWG revealed an increase among the subjects who were vitamin D-deficient in early pregnancy, and this was greater than that found for the non-deficient subjects, the difference was not statistically significant ( $p = 0.179$ ) (**Figure 6.6**). Percentage change in body fat failed to demonstrate any significant difference between the vitamin D-deficient and non-deficient groups ( $10.6 \pm 26.8$  vs.  $8.2 \pm 14.2$ ,  $p = 0.560$ ).

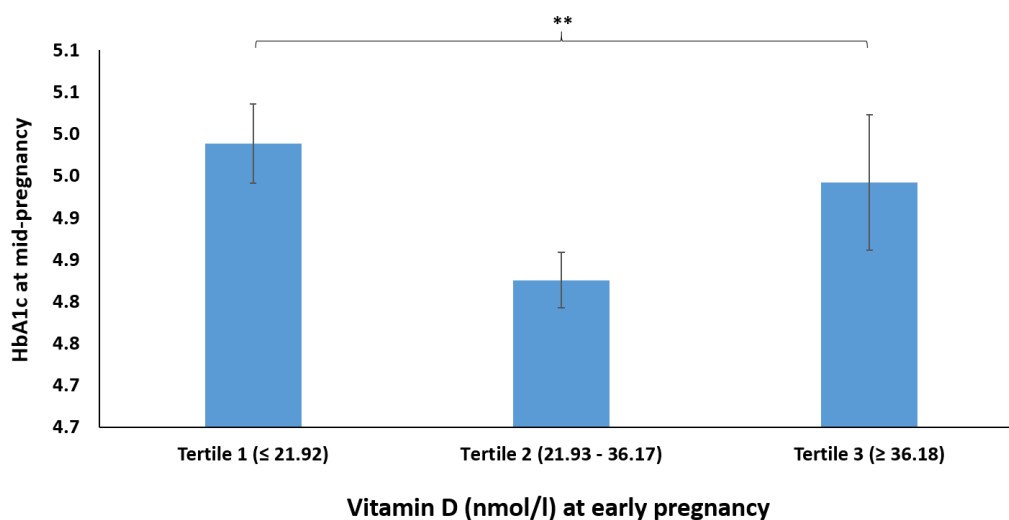




**Figure 6.6** Comparison of GWG between vitamin D-deficient and non-deficient subjects, during early pregnancy. The values show the percentage of women with excessive and non-excessive weight gain by mid-pregnancy, in both the vitamin D-deficient and non-deficient groups in early pregnancy. Pearson's chi-square test was applied.

### 6.3.2.1 Glycaemic Markers with vitamin D status in Early Pregnancy

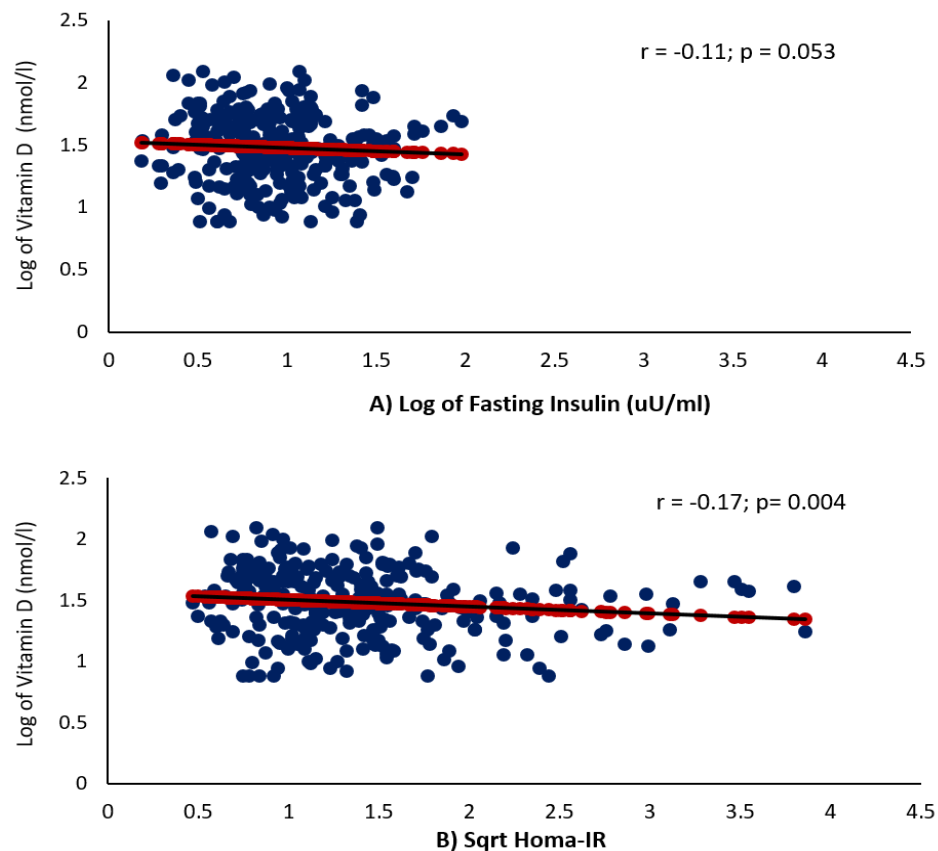
Fasting glucose among the pregnant women, who were deficient in vitamin D [4.5 mmol/L (4.0 - 5.0)] during early pregnancy, was higher than in the non-deficient subjects [4.2 mmol/L (3.9-4.6);  $p = 0.002$ ]. Negative correlations were found between early pregnancy vitamin D levels and fasting glucose levels ( $r = -0.12$ ,  $p = 0.046$ ) and HbA1c ( $r = -0.24$ ,  $p < 0.001$ ). Likewise, HbA1c (%) at mid-pregnancy among the vitamin D-deficient subjects ( $4.9 \pm 0.4$ ), during early pregnancy, was higher than for the non-deficient subjects ( $4.8 \pm 0.4$ ;  $p = 0.094$ ). This comparison reached a level of significance when HbA1c was compared according to vitamin D tertiles in early pregnancy, as illustrated in **Figure 6.7**. HOMA- $\beta$  was higher among the non-deficient women in early pregnancy [246.9 (147.9-367.0)] compared with the deficient subjects [175.9 (102.2-330.9),  $p = 0.033$ ], but lost significance after adjusting for age and BMI ( $p = 0.103$ ) (**Table 6.1**).



**Figure 6.7** HbA1c levels at mid-pregnancy, per vitamin D tertiles of the participants in early pregnancy. The values show the mean  $\pm$  SD of HbA1c, with vitamin D early pregnancy tertiles. P-value: \*\* denotes  $p < 0.01$ , obtained via ANOVA. Significance for HbA1c was only between tertile 1 and tertile 3.

### 6.3.2.2 Glycaemic Markers with vitamin D status in Mid-pregnancy

Mid-pregnancy vitamin D levels, fasting glucose, and HbA1c values were significantly higher among the vitamin D-deficient subjects, compared to their non-deficient counterparts after adjustment ( $p = 0.049$  and  $p = 0.019$ , respectively). Negative correlations between vitamin D and fasting glucose ( $r = -0.19$ ,  $p = 0.001$ ) and HbA1c ( $r = -0.22$ ,  $p < 0.001$ ) were observed at mid-pregnancy. Fasting insulin and HOMA-IR were higher in the vitamin D-deficient group, compared to the non-deficient group ( $p = 0.043$  and  $p = 0.020$ , respectively). The significance after adjustment for BMI and age was borderline ( $p = 0.054$  and  $p = 0.055$ ). Negative correlations were also observed between vitamin D levels and fasting insulin levels ( $r = -0.11$ ,  $p = 0.053$ ), and HOMA-IR ( $r = -0.17$ ,  $p = 0.004$ ), at mid-pregnancy (**Figure 6.8**). **Table 6.1** shows a comparison between GDM markers at mid-pregnancy, in relation to vitamin D status during early and mid-pregnancy.



**Figure 6.8** Correlations between the log of vitamin D (nmol/L) at mid-pregnancy, versus (A) the log of fasting insulin (Uu/mL) and (B) the Sqrt of HOMA-IR.

**Table 6.1 Biochemical GDM markers among vitamin D-deficient and non-deficient women during early and mid-pregnancy**

Mid-Pregnancy Parameters	Vitamin D Status in Early Pregnancy		p-value	*p-value
	Non-Deficient (N=67)	Deficient (N=230)		
<b>Fasting glucose (mmol/L) #</b>	4.2 (3.9-4.6)	4.5 (4.0-5.0)	<b>0.011</b>	<b>0.002</b>
1-hr glucose (mmol/L) #	7.4 (6.3-8.8)	7.5 (5.9-9.1)	0.949	0.984
2-hr glucose (mmol/L) #	6.1 (5.6-7.3)	6.3 (5.3-7.5)	0.908	0.603
Fasting insulin (uU/mL) #	9.7 (5.4-13.5)	7.6 (5.0-12.1)	0.168	0.232
2-hr insulin (uU/mL) #	43.3 (19.9-69.7)	41.0 (25.9-61.7)	0.709	0.313
HOMA-IR #	1.8 (0.9-2.8)	1.6 (0.9-2.6)	0.717	0.885
HOMA-β #	246.9 (147.9-367.0)	175.9 (102.2-330.9)	0.033	0.103
HbA1c (%)	4.8 ± 0.4	4.9 ± 0.4	0.167	0.094
<b>Vitamin D Status Mid-pregnancy</b>				
	Non-Deficient (N=50)	Deficient (N=247)		
<b>Fasting glucose (mmol/L) #</b>	4.3 (3.9-4.6)	4.5 (4.0-5.0)	<b>0.026</b>	<b>0.046</b>
1-hr glucose (mmol/L) #	8.0 (6.2-8.9)	7.4 (5.9-9.0)	0.734	0.752
2-hr glucose (mmol/L) #	6.1 (5.2-7.3)	6.3 (5.3-7.5)	0.378	0.316
Fasting insulin (uU/mL) #	6.4 (4.3-11.6)	8.4 (5.4-14.0)	0.043	0.054
2-hr insulin (uU/mL) #	43.8 (26.3-64.6)	41.0 (25.3-61.7)	0.682	0.648
HOMA-IR #	1.2 (0.7-2.3)	1.7 (1.0-3.1)	0.020	0.055
HOMA-β #	208.7 (118.1-367.0)	193.8 (104.0-337.6)	0.577	0.782
<b>HbA1c (%)</b>	4.8 ± 0.4	4.9 ± 0.5	<b>0.023</b>	<b>0.019</b>

**Note:** Data are presented as mean and standard deviation for normally distributed variables, while the median, 1<sup>st</sup> and 3<sup>rd</sup> quartiles are presented for non-normally distributed variables. # indicates non-normally distributed variables; the p-value for the mean differences were obtained using an independent sample t-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables; \* indicates p-values adjusted for age and BMI at 1<sup>st</sup> visit.

### **6.3.3 Vitamin D Deficiency and the Risk of Obesity, Hypertension, and Metabolic Syndrome**

#### ***6.3.3.1 Early Pregnancy***

Vitamin D deficiency during early pregnancy did not pose any risk to the lipid profile, metabolic syndrome, obesity, or HTN by mid-pregnancy.

#### ***6.3.3.2 Mid-pregnancy***

Vitamin D deficiency at mid-pregnancy was an independent risk factor for metabolic syndrome (OR = 5.79, 95% CI 1.48-22.66,  $p = 0.012$ ). Vitamin D deficiency at mid-pregnancy increased the risk of low HDL-cholesterol (OR = 2.58, 95%, CI 0.99-6.72,  $p = 0.049$ ), but no risks were posed for obesity or HTN (**Table 6.2**).

**Table 6.2 Vitamin D deficiency at mid-pregnancy, and its association with obesity, HTN and metabolic syndrome**

Diseases at Mid-Pregnancy	Unadjusted		Model 1		Model 2		Model 3		Model 4		Model 5	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
<b>Vitamin D Deficiency at Mid-pregnancy as a Risk Factor</b>												
Obesity	0.88 (0.51-1.53)	0.660	0.87 (0.50-1.53)	0.633	1.06 (0.53-2.10)	0.871	1.21 (0.59-2.51)	0.602	1.10 (0.51-2.37)	0.800	1.04 (0.48-2.25)	0.926
HTN	1.28 (0.27-6.07)	0.759	1.14 (0.24-5.54)	0.856	1.04 (0.19-5.73)	0.963	1.56 (0.24-10.27)	0.643	1.21 (0.17-8.78)	0.851	1.45 (0.20-10.73)	0.716
<b>Low HDL-cholesterol</b> ( $<1.03$ mmol/l)	1.61 (0.85-3.05)	0.142	1.65 (0.86-3.15)	0.132	2.16 (0.94-4.95)	0.069	2.12 (0.91-4.93)	0.081	2.70 (1.06-6.87)	0.037	2.58 (0.99-6.72)	<b>0.049</b>
Total cholesterol-HDL ratio $> 3.5$	1.16 (0.64-2.09)	0.625	1.21 (0.67-2.22)	0.521	2.00 (0.96-4.17)	0.065	2.03 (0.94-4.40)	0.074	2.04 (0.87-4.76)	0.100	1.98 (0.80-4.87)	0.138
TG $\geq 1.7$ (mmol/L)	1.42 (0.81-2.49)	0.218	1.39 (0.78-2.46)	0.262	1.21 (0.62-2.35)	0.576	1.24 (0.62-2.47)	0.548	1.33 (0.64-2.78)	0.448	1.29 (0.61-2.74)	0.504
<b>Metabolic Syndrome</b>	1.54 (0.70-3.37)	0.280	1.70 (0.73-3.96)	0.216	3.60 (1.04-12.45)	0.043	3.72 (1.07-12.95)	0.039	4.84 (1.29-18.15)	0.019	5.79 (1.48-22.66)	<b>0.012</b>

**Note:** Data are presented as slope, odds ratio (OR) and 95% confidence interval (CI) for OR, using logistic regression analysis, taking vitamin D deficiency as a risk factor and disease as dependent variable at visit 1; Model 1: Age and BMI at visit 1; Model 2: Model 1 + parity, education, employment, residence in North Riyadh, past GDM, family history (obesity, DM and GDM). Adjustment 3: Model 2 + total GWG based on weight at visit 1 + change in fat %; Model 4: Model 3 + sun exposure by visit 1, season at visit 1, vitamin D intake by visit 1, multivitamin by visit 1; Model 5: Model 4 + physical activity by visit 1. HTN indicates Hypertensi

## 6.3.4 Predictors of GDM

### 6.3.4.1 Predictors of GDM in Early Pregnancy

Data for predictors of GDM among the subjects during early pregnancy are shown in **Table 6.3**. Previous history of GDM (OR 11.13 95%, CI 3.10-39.96,  $p < 0.001$ ), vitamin D deficiency (OR 3.97, 95%, CI 1.12-14.15,  $p < 0.033$ ), high HbA1c (OR 3.06, 95% CI 1.32-7.07,  $p = 0.037$ ), and low HDL-cholesterol (OR 2.11, 95% CI 1.01-4.39,  $p < 0.046$ ) during early pregnancy posed a significant risk for the development of GDM.

Blood samples from subjects in early pregnancy that were collected during the summer season (OR 2.15, 95% CI 0.99-4.67,  $p = 0.053$ ) showed an increased risk of GDM, but this was not statistically significant. Before adjustments were made, pre-pregnancy BMI (OR 1.06, 95% CI 1.02-1.11,  $p = 0.003$ ), early pregnancy obesity (OR 1.72, 95% CI 1.04-2.85,  $p = 0.033$ ), a family history of diabetes (OR 1.87, 95% CI 1.02-3.44,  $p = 0.044$ ), and random blood glucose (OR 1.36, 95% CI 1.08-1.71,  $p = 0.008$ ), were also associated with an increased risk of GDM. Random blood glucose and pre-pregnancy BMI remained significant, even after adjusting for age and BMI at the 1<sup>st</sup> visit. In contrast, low-intensity physical activity during early pregnancy was found to provide some protection against the development of GDM, even after adjusting for age and BMI (OR 0.56, 95% CI 0.33-0.97,  $p = 0.039$ ).

### 6.3.4.2 Associated Factors of GDM at Mid-pregnancy

Data on the associated factors of GDM among the subjects' mid-pregnancy are described in **Table 6.3**. The presence of metabolic syndrome (OR 57.61, 95%, CI 12.64-262.64,  $p < 0.001$ ), obesity (OR 3.59 95%, CI 1.04-12.40,  $p = 0.043$ ), and high HbA1c (OR 2.29, 95% CI 1.01-5.19,  $p = 0.047$ ) were observed as independent variables, associated with an increased risk of developing GDM at mid-pregnancy. Although mid-pregnancy hypertriglyceridemia increased the risk of GDM (OR 1.84, 95% CI 1.08-3.15,  $p = 0.025$ ), the significance was lost after adjustment. Multivitamin supplementation, however, was noted to confer some protection against the development of GDM at mid-pregnancy (OR 0.38, 95% CI 0.18-0.81,  $p = 0.013$ ).

**Table 6.3 Predictors of GDM among the subjects during (A) Early pregnancy and (B) Mid-pregnancy**

	Unadjusted		Model 1		Model 2		Model 3		Model 4		Model 5	
<i>(A) Early Pregnancy Parameters</i>	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Age	1.04 (0.99-1.09)	0.099	1.03 (0.98-1.08)	0.287	1.02 (0.96-1.09)	0.490	1.02 (0.95-1.09)	0.545	1.02 (0.95-1.10)	0.524	1.02 (0.94-1.09)	0.690
Multiparity	1.65 (0.96-2.81)	0.068	1.37 (0.77-2.44)	0.290	1.23 (0.61-2.48)	0.565	1.28 (0.62-2.63)	0.502	1.22 (0.56-2.63)	0.617	1.31 (0.59-2.89)	0.511
Pre-pregnancy BMI (Kg/m <sup>2</sup> )	1.06 (1.02-1.11)	0.003	1.05 (1.01-1.10)	0.021	1.04 (0.98-1.10)	0.191	1.03 (0.97-1.09)	0.308	1.04 (0.98-1.11)	0.219	1.05 (0.98-1.12)	0.141
Obesity	1.72 (1.04-2.85)	0.033	1.02 (0.43-2.43)	0.970	1.53 (0.78-2.98)	0.216	1.44 (0.71-2.93)	0.317	1.45 (0.69-3.05)	0.324	1.65 (0.77-3.56)	0.201
University graduate or postgraduate	0.67 (0.41-1.09)	0.108	0.70 (0.42-1.17)	0.173	0.72 (0.36-1.43)	0.346	0.67 (0.33-1.34)	0.254	0.63 (0.30-1.31)	0.215	0.65 (0.31-1.38)	0.265
Employment	1.05 (0.63-1.76)	0.839	1.01 (0.60-1.72)	0.964	1.21 (0.61-2.43)	0.587	1.37 (0.67-2.79)	0.383	1.55 (0.74-3.27)	0.250	1.71 (0.79-3.69)	0.176
<b>Previous GDM</b>	<b>14.64 (4.79-44.69)</b>	<b>&lt; 0.001</b>	<b>14.93 (4.70-47.42)</b>	<b>&lt; 0.001</b>	<b>11.60 (3.36-39.99)</b>	<b>&lt; 0.001</b>	<b>11.42 (3.31-39.41)</b>	<b>&lt; 0.001</b>	<b>10.56 (3.04-36.61)</b>	<b>&lt;0.001</b>	<b>11.13(3.10-39.96)</b>	<b>&lt;0.001</b>
Family history of GDM	1.32 (0.75-2.31)	0.340	1.29 (0.73-2.29)	0.377	0.99 (0.50-1.99)	0.983	0.95 (0.48-1.97)	0.975	0.90 (0.43-1.90)	0.785	0.80 (0.37-1.73)	0.576
Family history of obesity	0.84 (0.34-2.09)	0.704	0.78 (0.30-2.00)	0.596	0.79 (0.28-2.21)	0.654	0.71 (0.25-2.02)	0.516	0.74 (0.25-2.18)	0.578	0.70 (0.23-2.12)	0.530
Family history of DM	1.87 (1.02-3.44)	0.044	1.64 (0.88-3.05)	0.123	1.28 (0.64-2.53)	0.487	1.24 (0.61-2.51)	0.547	1.44 (0.68-3.02)	0.341	1.47 (0.67-3.21)	0.332
<b>HbA1c (%)</b>	<b>2.56 (1.50-4.36)</b>	<b>0.001</b>	<b>2.28 (1.32-3.95)</b>	<b>0.003</b>	<b>2.85 (1.34-6.06)</b>	<b>0.007</b>	<b>2.66 (1.23-5.76)</b>	<b>0.013</b>	<b>2.83 (1.27-6.35)</b>	<b>0.011</b>	<b>3.06 (1.32-7.07)</b>	<b>0.009</b>
Random glucose (mmol/L)	1.45 (1.17-1.81)	0.001	1.36 (1.08-1.71)	0.008	1.30 (0.97-1.73)	0.083	1.43 (1.03-1.98)	0.031	1.37 (0.98-1.91)	0.068	1.33 (0.93-1.89)	0.116
TG ( $\geq$ 1.7 mmol/L)	1.65 (0.99-2.74)	0.054	1.47 (0.87-2.48)	0.151	1.21 (0.59-2.49)	0.599	1.11 (0.53-2.30)	0.790	1.17 (0.54-2.52)	0.693	1.02 (0.45-2.29)	0.972
Total cholesterol HDL ratio > 3.5	1.31 (0.78-2.20)	0.317	1.18 (0.69-2.01)	0.547	1.47 (0.73-2.93)	0.279	1.41 (0.69-2.91)	0.351	1.53 (0.72-3.24)	0.269	1.30 (0.60-2.84)	0.511
<b>Low HDL-cholesterol (&lt; 1.03 mmol/L)</b>	<b>1.44 (0.89-2.34)</b>	<b>0.140</b>	<b>1.47 (0.90-2.42)</b>	<b>0.125</b>	<b>1.83 (0.97-3.47)</b>	<b>0.063</b>	<b>1.91 (0.99-3.67)</b>	<b>0.055</b>	<b>2.12 (1.04-4.32)</b>	<b>0.040</b>	<b>2.11 (1.01-4.39)</b>	<b>0.046</b>
HTN	0.35 (0.08-1.60)	0.174	0.22 (0.05-1.10)	0.065	0.26 (0.03-2.49)	0.244	0.26 (0.03-2.50)	0.245	0.23 (0.02-2.23)	0.205	0.26 (0.03-2.62)	0.254
Vitamin D intake (> 600 IU/day)	0.43 (0.05-3.72)	0.441	0.46 (0.05-4.09)	0.489	0.87 (0.07-11.03)	0.916	0.72 (0.06-9.45)	0.801	0.69 (0.05-9.18)	0.781	0.60 (0.04-8.17)	0.703
Use of multivitamins	1.53 (0.81-2.89)	0.194	1.36 (0.70-2.65)	0.369	0.86 (0.37-2.05)	0.739	0.94 (0.39-2.25)	0.889	0.86 (0.34-2.17)	0.742	0.91 (0.33-2.48)	0.846
Low intensity PA ( $\geq$ 210 mints/wk)	0.61 (0.36-1.03)	0.064	0.56 (0.33-0.97)	0.039	0.56 (0.27-1.14)	0.110	0.60 (0.28-1.29)	0.192	0.58 (0.26-1.29)	0.183	----	----
Blood sample collection (Summer)	1.60 (0.97-2.63)	0.064	1.78 (1.07-2.98)	0.027	1.91 (0.99-3.68)	0.054	1.96 (1.00-3.85)	0.049	2.11 (1.02-4.37)	0.045	2.15 (0.99-4.67)	0.053
<b>Vitamin D deficiency at 1<sup>st</sup> visit</b>	<b>4.43 (1.82-10.79)</b>	<b>0.001</b>	<b>4.63 (1.87-11.46)</b>	<b>0.001</b>	<b>3.71 (1.18-11.65)</b>	<b>0.025</b>	<b>3.94 (1.24-12.46)</b>	<b>0.020</b>	<b>4.40 (1.33-14.54)</b>	<b>0.015</b>	<b>3.97 (1.12-14.15)</b>	<b>0.033</b>



	Unadjusted		Model 1		Model 2		Model 3		Model 4		Model 5	
<b>(B) Mid-pregnancy Parameters</b>	<b>OR (95% CI)</b>	<b>P-value</b>	<b>OR (95% CI)</b>	<b>P-value</b>	<b>OR (95% CI)</b>	<b>P-value</b>	<b>OR (95% CI)</b>	<b>P-value</b>	<b>OR (95% CI)</b>	<b>P-value</b>	<b>OR (95% CI)</b>	<b>P-value</b>
Vitamin D deficiency 2 <sup>nd</sup> visit	2.00 (1.06-3.76)	0.033	2.00 (1.05-3.82)	0.034	1.89 (0.88-4.06)	0.101	1.93 (0.89-4.21)	0.097	1.88 (0.85-4.17)	0.122	1.98 (0.89-4.41)	0.096
<b>Obesity 2<sup>nd</sup> visit</b>	<b>2.03 (1.25-3.32)</b>	<b>0.005</b>	<b>2.11 (0.94-4.75)</b>	<b>0.070</b>	<b>3.40 (1.19-9.72)</b>	<b>0.022</b>	<b>4.63 (1.50-14.31)</b>	<b>0.008</b>	<b>4.21 (1.29-13.71)</b>	<b>0.017</b>	<b>3.59 (1.04-12.40)</b>	<b>0.043</b>
GWG (excessive)	0.92 (0.56-1.52)	0.744	0.88 (0.52-1.49)	0.623	0.99 (0.71-1.38)	0.956	1.08 (0.71-1.66)	0.710	1.11 (0.71-1.74)	0.639	1.04 (0.66-1.65)	0.858
Δ Body fat (%)	1.00 (1.00-1.01)	0.952	1.00 (0.99-1.02)	0.492	0.99 (0.96-1.01)	0.237	0.99 (0.96-1.01)	0.281	0.98 (0.96-1.01)	0.206	0.99 (0.96-1.01)	0.255
<b>HbA1c (%)</b>	<b>3.13 (1.83-6.00)</b>	<b>&lt; 0.001</b>	<b>2.89 (1.58-5.29)</b>	<b>0.001</b>	<b>2.51 (1.19-5.30)</b>	<b>0.016</b>	<b>2.44 (1.15-5.19)</b>	<b>0.020</b>	<b>2.48 (1.12-5.50)</b>	<b>0.026</b>	<b>2.29 (1.01-5.19)</b>	<b>0.047</b>
TG (≥ 1.7 mmol/l)	1.84 (1.08-3.15)	0.025	1.69 (0.98-2.91)	0.061	1.96 (0.98-3.93)	0.057	1.84 (0.91-3.75)	0.092	1.85 (0.87-3.92)	0.108	1.70 (0.78-3.67)	0.179
<b>Metabolic syndrome</b>	<b>12.45 (5.9-25.97)</b>	<b>&lt; 0.001</b>	<b>14.46 (6.36-32.87)</b>	<b>&lt; 0.001</b>	<b>39.61 (11.11-141.31)</b>	<b>&lt; 0.001</b>	<b>42.08 (11.44-154.77)</b>	<b>&lt; 0.001</b>	<b>41.45 (10.51-163.52)</b>	<b>&lt;0.001</b>	<b>57.61 (12.64-262.64)</b>	<b>&lt;0.001</b>
HTN	1.67 (0.50-5.61)	0.409	1.27 (0.34-4.71)	0.720	1.87 (0.39-8.99)	0.436	1.62 (0.33-7.84)	0.551	1.47 (0.29-7.33)	0.642	1.36 (0.26-7.12)	0.713
Vitamin D intake (>600 IU/day)	1.65 (0.36 - 7.57)	0.517	1.82 (0.39-8.49)	0.446	0.59 (0.06-5.63)	0.648	0.56 (0.06-5.39)	0.614	0.52 (0.05-5.32)	0.580	0.44 (0.04-4.53)	0.486
Calcium intake (>1000 mg/day)	1.25 (0.54 - 2.85)	0.604	1.23 (0.53-2.82)	0.632	1.51 (0.62-3.69)	0.366	1.47 (0.59-3.65)	0.405	1.45 (0.54-3.91)	0.465	1.40 (0.52-3.79)	0.512
<b>Multivitamin supplementation</b>	<b>0.61 (0.34-1.10)</b>	<b>0.099</b>	<b>0.59 (0.33-1.07)</b>	<b>0.084</b>	<b>0.44 (0.22-0.87)</b>	<b>0.019</b>	<b>0.42 (0.21-0.85)</b>	<b>0.015</b>	<b>0.47 (0.23-0.98)</b>	<b>0.043</b>	<b>0.38 (0.18-0.81)</b>	<b>0.013</b>
Low intensity PA (≥210 mints/wk)	0.76 (0.45-1.28)	0.298	0.70 (0.41-1.20)	0.198	0.67 (0.33-1.36)	0.269	0.80 (0.37-1.71)	0.559	0.89 (0.41-1.93)	0.760	1.04 (0.46-2.38)	0.918

**Note:** Data are presented as slope, odds ratio (OR), and 95% confidence interval (CI) for OR using logistic regression analysis, and taking GDM as a dependent variable against potential risk factors at visit 1; Model 1: Age and BMI at visit 1; Model 2: Model 1 + parity, education, employment, residence in South Riyadh, past GDM, family history (obesity, DM and GDM). Model 3: Model 2 + total GWG as at visit 1 + changes in fat %; Model 4: Model 3 + sun exposure by visit 1, season at visit 1, vitamin D intake by visit 1, multivitamin intake by visit 1; Model 5: Model 4 + physical activity (PA) by visit 1.

## 6.4 Discussion

In spite of the high levels of vitamin D deficiency observed in early pregnancy, and at delivery in Saudi Arabia, this is the first study conducted in the above context to examine changes in levels of vitamin D deficiency, and the influence of these on maternal and birth outcomes. In this prospective study, associations between vitamin D status and GDM risk have been explored, following adjustment for both vitamin D and GDM risk factors. These studies indicate that low vitamin D levels in early and mid-pregnancy are associated with increased risk of GDM development. Furthermore, the fact that vitamin D deficiency is observed to be independently associated with metabolic syndrome, and an increased risk of low-HDL cholesterol, suggests that it is not only associated with abnormal glycaemia, but also increases cardio-metabolic risk.

This study specifically reveals that vitamin D deficiency in early pregnancy is associated with up to 3.97 times greater risk of developing GDM, after adjusting for potential confounding factors, including lifestyle factors. In three different studies, low vitamin D concentration during the first trimester of pregnancy showed an association with a 2.66, 2.21, and 1.9 times higher risk of developing GDM at 24-28 weeks, after adjusting for classical risk factors, such as BMI, ethnicity, season, and age (Zhang et al., 2008; Parlea et al., 2012; Arnold et al., 2015). As such, the risk posed by low blood levels of 25(OH)D among the pregnant women in the present study was significantly higher than the previously reported hypovitaminosis D-related risk of developing GDM. Moreover, the higher risk of developing GDM associated with vitamin D deficiency, in the present study, was determined after adjusting for risk factors for both vitamin D deficiency and GDM. This finding is consistent with one Canadian study reporting vitamin D deficiency as an important determinant for the development of GDM, after adjusting for vitamin D confounding factors, such as the season, sun exposure, dietary intake of vitamin D, and PTH levels, along with adjusting for other GDM risk factors, such as maternal age, ethnicity, parity, and obesity (Lacroix et al., 2014). Five independent meta-analyses of observational studies have reported a 38-61% higher risk of GDM among vitamin D-deficient pregnant women (Poel et al., 2012; Aghajafari et al., 2013; Wei et al., 2013; Zhang et al., 2015; Lu et al., 2016). Although

there are data suggesting that vitamin D deficiency during early pregnancy is not a significant risk factor for developing future GDM (Baker et al., 2012), 73% of the participants investigated had sufficient levels of vitamin D. Multivitamin or vitamin D supplementation, along with the fact of residing in geographical locations that favour an adequate vitamin D status, may have contributed to the observed discrepancy.

In this current study, vitamin D levels during early and mid-pregnancy were found to negatively correlate with fasting glucose and HbA1c at mid-pregnancy. This finding is consistent with a study by Makgoba et al. (2011), which also reported a negative correlation between first-trimester vitamin D and second-trimester GDM markers, including fasting glucose and 2-hour OGTT. Furthermore, after adjusting for confounding factors, maternal 25(OH)D inadequacy in the second trimester was shown to be associated with higher fasting glucose concentrations, but not 2-hour OGTT concentrations (Loy et al., 2015). No association was observed between early vitamin D deficiency and OGTT values in the present study. This suggests that 2-hour OGTT may be less likely to be influenced by vitamin D concentration, although the variability of the latter is more apparent than that of fasting glucose. In addition, it has been stated that insulin resistance can influence 1- and 2-hour postprandial glucose levels (McLeod et al., 2012). As such, it is possible that 25(OH)D may not affect glucose metabolism via modulation of insulin sensitivity, but could do so through other mechanisms, such as modifying pancreatic  $\beta$ -cell function, or cytokine production (Pittas et al., 2007). Furthermore, a negative correlation between vitamin D in the first (Alamolhoda et al., 2010; Makgoba et al., 2011) and second half of pregnancy (Lau et al., 2011; El Lithy et al., 2014), and HbA1c in mid-pregnancy has previously been reported. The noted association between vitamin D and HbA1c points to a potential interaction between vitamin D and glycaemic control during pregnancy.

A relatively small number of studies have assessed the relationship between vitamin D and insulin in pregnancy, but this current study reveals a correlation between mid-pregnancy vitamin D deficiency and impaired insulin sensitivity, thus confirming the earlier studies (Clifton-Bligh et al., 2008; Farrant et al., 2008; McLeod et al., 2012; Wang et al., 2012; Mutlu et al., 2015).

It is important to stress that the limited studies carried out have not detected an association between GDM or its markers, and vitamin D deficiency (Parildar et al., 2013; Kramer et al., 2014; Park et al., 2014). This is partly due to the fact that the studies that have addressed the topic of GDM, did not explore its markers, so their results have been vague with regard to glycaemia (Schneuer et al., 2014; Flood-Nichols et al., 2015; Rodriguez et al., 2015). Contradictory findings may also have arisen, due to the small number of GDM subjects participating in the studies mentioned above, or to the high levels of heterogeneity between the studies themselves. This can be attributed to different methodological factors, ethnicity, study design, sample size, trimester of pregnancy, or lack of adjustment for confounding factors, including vitamin D supplementation and seasonal variation. Moreover, the heterogeneity of dietary factors and differences in socio-economic status, as well as low prevalence of vitamin D deficiency are further factors potentially impacting the results (Burris et al., 2012; Schneuer et al., 2014; Loy et al., 2015).

Interestingly, the current study revealed links between vitamin D related lifestyle factors, linking to GDM and its markers. Mid-pregnancy, the percentage of women covering the whole of their bodies and those experiencing less exposure to sunlight, particularly at noon, was higher among the GDM subjects, compared to those with no GDM. This is because sunlight exposure stimulates vitamin D synthesis in the skin (Holick, 2004), therefore less exposure to sunlight will result in decreased vitamin D production during mid-pregnancy, which may in turn contribute to the development of GDM (Maghbooli et al., 2008; Zuhur et al., 2013; Jain et al., 2015). Furthermore, it was interesting to note in the current study that seasonal changes (summer vs. winter) in early pregnancy showed an increasing trend towards GDM prevalence. This is similar to other recent, but limited, research (Moses et al., 2016; Verburg et al., 2016).

Vitamin D and calcium intake during early and mid-pregnancy significantly correlated with fasting glucose in this instance. This is reminiscent of a recent study that found a positive association between GDM, and higher vitamin D and calcium intake (Meinilä et al., 2015). The increase may be explained by the greater consumption of high-fat dairy products during pregnancy, and it is a relationship that should be studied further.

Moreover, multivitamin supplementation by mid-pregnancy appears to confer some protection against the development of GDM, in the current study. Vitamin D and calcium supplementation in pregnant women have a beneficial effect on glycaemia (Asemi et al., 2013). Supplementation with vitamins C, E, and B12, have also been shown to be associated with a beneficial effect on glycaemic control (Reunanen et al., 1998; Song et al., 2011; Sukumar et al., 2015; Tabatabaei-Malazy et al., 2015), and have been indicated in decreasing the risk of developing future diabetes. However, it remains to be determined whether a single component, or a combination of various vitamins and minerals in multivitamin preparations, confers protection against T2DM (Ford, 2001). Moreover, details regarding the type, dosage, frequency, and duration of vitamin use was unavailable from the participants, in the present study.

Aside from the above, low vitamin D levels positively associated with metabolic syndrome and low HDL-cholesterol, independent of several potential confounding factors, including obesity, physical activity, age, and parity. Asemi et al. (2013) reported that vitamin D supplements could induce a significant reduction in total serum cholesterol and LDL-cholesterol concentrations. Moreover, hypovitaminosis D has been shown to increase the risk of components of metabolic syndrome in pregnancy, such as obesity (Karras et al., 2016), high levels of triglycerides (Rodriguez-Rodriguez et al., 2011), low HDL-cholesterol (Makgoba et al., 2011), fasting blood glucose level (Loy et al., 2015), and gestational HTN (Bodnar et al., 2007a). A meta-analysis of the non-pregnant population demonstrated an inverse correlation between vitamin D levels and the risk of metabolic syndrome (Ju et al., 2013).

Confirming the results presented in Chapter 5, the present study confirms that a past history of GDM and obesity are strong predictors of future GDM development (Kautzky-Willer et al., 2008; Al-Rubeaan et al., 2014; Erem et al., 2015; Qing et al., 2016). In addition, the present study demonstrates that high HbA1c levels in early pregnancy pose a three-fold risk during early pregnancy, and 2.29 times risk during mid-pregnancy, for the development of GDM. High HbA1c levels at 18-22 and 32-36 weeks of pregnancy are associated with 11.4 and 56 times the risk of developing GDM, respectively (Odsæter et al., 2016). Although HbA1c as a risk factor for GDM

development has not been investigated extensively, a few studies have cited HbA1c as a risk factor, but none adjusted for lifestyle factors as independent risk factors. However, the present study aimed to address this and did perform adjustments for lifestyle factors in order to ascertain their independent risk effects. Although HbA1c may be an important predictor of GDM, as a single entity it does not seem to be a robust criterion for diagnosing GDM (Agarwal et al., 2005; Rajput et al., 2012; Sevket et al., 2014; Odsæter et al., 2016).

Furthermore, a few but inconsistent studies have focused on the role of early pregnancy lipid profiles and GDM risk (Enquobahrie et al., 2005; Li et al., 2015; Wang et al., 2016). This current study revealed that within the lipid profile, only low HDL-cholesterol in early pregnancy appeared to be an independent risk factor of GDM, which remained significant after adjusting for confounding factors. This observation is consistent with the findings of previous studies reporting low levels of HDL-cholesterol in the first trimester; these being associated with a higher risk of future GDM development, even after adjusting for confounding factors (Savvidou et al., 2010; Makgoba et al., 2011; Li et al., 2015).

T2DM, GDM, and metabolic syndrome all share similar risk factors, particularly against the backdrop of similar genetic susceptibilities (Ben-Haroush et al., 2004). Therefore, it is assumed that their pathogenesis is indistinct, with one preceding the other, and many components of the metabolic syndrome predicting GDM. It has been proposed that GDM is a phase of metabolic syndrome that refers to a combination of high insulin levels, insulin resistance, obesity, dyslipidaemia, hypertension, and T2DM, or impaired glucose tolerance (Ben-Haroush et al., 2004). Metabolic syndrome was in fact found to incur 57.61 times the risk of developing GDM by mid-pregnancy, which could be ascribed to significantly higher components of metabolic syndrome here, including obesity, TG, fasting glucose, fasting insulin, and HOMA-IR, in the GDM group. However, limited studies have considered metabolic syndrome with GDM risk during normal pregnancies (Bartha et al., 2008; Negrato et al., 2008). Metabolic syndrome is certainly linked to a worsening of glucose homeostasis at mid-pregnancy, with the subsequent development of GDM (Negrato et al., 2008), and was detected in

45.7% of the pregnant women in the present study, which was significantly higher than the 10% prevalence rate reported previously by Bartha et al. (2008).

In conclusion, the current study has verified that hypovitaminosis pertaining to vitamin D, during the first trimester of pregnancy is a significant risk factor for developing GDM, even after adjusting for lifestyle factors. The findings of this study, therefore, put forward additional arguments concerning the effect of vitamin D on alterations to lipid profiles and metabolic syndrome, thus emphasising the need for further investigation, so that these potentially modifiable complications can be detected in a timely manner, leading to better pregnancy outcomes.

## **Chapter 7**

### **Final Discussion**



## **7.1 Discussion**

### **7.1.1 Vitamin D Status Among Pregnant Saudi Women**

During pregnancy, a number of endogenous and exogenous factors, such as maternal blood glucose, insulin, and vitamin D concentration affect maternal and foetal homeostasis (Drever et al., 2010; Lukaszewski et al., 2013; Karras et al., 2014). Among the pregnant women included in the present study, 81% were found to have a vitamin D deficiency, which is higher than the prevalence rates reported in European and Western countries (Makgoba et al., 2011; Wei et al., 2012; Flood-Nichols et al., 2015). However, vitamin D deficiency rates of up to 98% among Asian pregnant women, indicates that these rates exhibit regional and ethnic variations (Narchi et al., 2010; Al Kalbani et al., 2011; Choi et al., 2015). Vitamin D deficiency among women, particularly during pregnancy, may adversely affect pregnancy outcomes (Lu et al., 2016). This emphasises the need to gain a better understanding of factors predisposing pregnant women to vitamin D deficiency, particularly in the initial stages of pregnancy.

### **7.1.2 Factors Determining Vitamin D Status Among Pregnant Saudi Women**

Exposure to sunlight is critical for adequate supplies of vitamin D, as 80% of vitamin D is derived from the skin reacting to sunlight (Holick, 2003). Limited exposure to sunlight is, therefore, considered an important predictor of vitamin D deficiency (Holick, 2004). Avoidance of sun exposure by spending a significant amount of time indoors has already been recognised as a major risk factor in the development of vitamin D deficiency (Xiang et al., 2013). The pregnant women in the present study were observed to experience inadequate sun exposure through being indoors, which was linked to extreme climatic conditions and the wearing of traditional clothing that covers the whole body. These factors are believed to contribute to vitamin D deficiency in the current context. Whole body coverage with traditional dress has been shown to pose a 17 times higher risk of vitamin D deficiency, and this reflects what has been observed in other Muslim countries (Ates et al., 2016; Karras et al., 2016).

Vitamin D intake from food was also found to be low in the sample studied here, with only 2.6% of the subjects ingesting more than the daily recommended intake. Since food sources provide less than 10% of the human body's daily vitamin D requirement, reliance solely on dietary sources is expected to result in hypovitaminosis D (Holick et al., 2012). Moreover, recent unhealthy diet rich in fat and carbohydrates and changes in eating habits in KSA may have contributed to lower dietary intake of vitamin D and calcium, especially in younger women (Al-Ghamdi et al., 2012; Al-Faris et al., 2015). In addition, successive and unplanned pregnancies in a community with a high prevalence of vitamin D deficiency may further aggravate the condition (Aly et al., 2013; Andersen et al., 2013). Accordingly, multiparity was also associated with vitamin D deficiency in the present study; due to depletion of the vitamin D reserves in the body especially if there is a lack of vitamin D supplementation or faulted dietary behaviors within the pregnancy spacing periods (Jensen et al., 2012). Pregnant housewives with low educational status, residing in low income localities were all consequently found to be vulnerable to vitamin D deficiency, reflecting a lack of awareness in this particular group.

The individuals with no vitamin D deficiency were also found to have a higher body fat percentage than the members of the vitamin D-deficient group. This could be explained by rapid dynamic changes in lipid metabolism during this period of their pregnancies (Lain & Catalano, 2007), thus triggering higher serum 25(OH)D concentrations. It is well known that fatty dairy products are rich in vitamin D, so it is highly likely that adequate levels of vitamin D were maintained by a higher dietary fat and vitamin D content in the non-deficient group. However, assessment of body fat during pregnancy, by measuring skinfold thickness is prone to error, due to pregnancy-related hormonal changes resulting in increased hydration of the connective tissues (Robertson, 1969). Despite this, estimation of body fat using skinfold thickness remains the best measure of percentage body fat, independent of the changes related to the presence of the foetus (Forsum et al., 1989; Widen & Gallagher, 2014). Nevertheless, although fat mass quantification is important for understanding vitamin D status during all stages of life, this could be less important in pregnancy compare with other risk

factors, including physical activity, which appears to be more relevant to vitamin D status (Bener et al., 2013; Simões et al., 2016).

To illustrate this, the physically active women in the present study were found to have adequate levels of vitamin D, with physical activity of 30 minutes/day being observed to be an independent predictor of 25(OH)D adequacy, even after adjusting for sun exposure. This observation is consistent with previously reported findings, suggesting that an active lifestyle will support adequate vitamin D levels (Kluczynski et al., 2011; Rodriguez et al., 2016). Correspondingly, it has been speculated that the maintenance of adequate vitamin D levels is most likely due to its mobilisation from the adipose tissues (Tzotzas et al., 2010).

Finally, low HDL-cholesterol levels, and the total cholesterol/HDL ratio have proved to be significant risk factors for hypovitaminosis D in early pregnancy. It has previously been shown that vitamin D concentration positively correlates with HDL cholesterol, even after adjusting for age, BMI, ethnicity, family history of diabetes, and cigarette smoking (Makgoba et al., 2011). Therefore, reduced HDL-cholesterol levels are not only associated with vitamin D deficiency, but also pose a significant risk for the development of cardiovascular disease, particularly among pregnant women (Boucher, 1998; Barter et al., 2007).

### **7.1.3 Vitamin D Status During Pregnancy**

The monitoring of vitamin D levels during pregnancy has been recommended for favourable pregnancy outcomes (Moon et al., 2015). It has been proposed that vitamin D status during early pregnancy is a reliable predictor of vitamin D concentrations later on in pregnancy, thus providing an opportunity to pre-empt, and avoid adverse effects of vitamin D deficiency in both the mother and the foetus (Choi et al., 2015). Although pregnant women with vitamin D deficiency during early pregnancy were found to exhibit an improvement in their vitamin D levels as their pregnancy progressed, they failed to achieve adequate levels of vitamin D by mid-pregnancy. Rising vitamin D levels with advancing pregnancy may have been attributed to increased production of

vitamin D by the growing placenta (Forsum et al., 1989; Widen & Gallagher, 2014). Moreover, genetic factors, vitamin D binding proteins, and high levels of oestrogens may have also be implicated in these rising concentrations during mid-pregnancy (Wang et al., 2010; Hedlund et al., 2013). The fact that 77% of the pregnant women displayed vitamin D deficiency at mid-pregnancy, indicates that endogenous sources of vitamin D fall short of providing sufficient amounts of vitamin D at such a time, but this may be compensated with increased physical activity and intake of vitamin D supplements.

#### **7.1.4 Prevalence and Risk Factors of GDM Among Pregnant Saudi Women**

Pregnancy has always been known as a diabetogenic state, characterised by overt insulin resistance and compensatory hyperinsulinaemia (Ryan, 2011). Coupled with high rates of obesity in the general population in Saudi Arabia, the risk of developing GDM is undoubtedly increased (Al-Rubeaan et al., 2014; Alfadhli et al., 2015). Similar observations were made in the present study, whereby 33% of pregnancies were found to be complicated by GDM.

A past history of GDM was associated with an 11 times higher risk of developing GDM in the present study, and this was among the strongest predictors of GDM. Other classical risk factors linked to GDM in the present study were family history of T2DM, pre-pregnancy obesity, obesity during early and mid-pregnancy, and physical inactivity. The subjects in the GDM group were obese, and thus cautious against gaining weight. Similar findings were derived from previous studies (Li et al., 2015; Hung & Hsieh, 2016). The GDM relationship with a higher cardio-metabolic profile was evident in this and earlier studies (Ryckman et al., 2015; Chitme et al., 2016).

#### **7.1.5 Novel Predictors of GDM in Pregnant Saudi Women**

The present study revealed some novel predictors of increased GDM risk in early pregnancy. For instance, random blood glucose and Hb1Ac levels were higher in the GDM women, compared to their counterparts without GDM. In fact, a high percentage

of Hb1Ac (the range in this study was from 4% - 6.4%) in early pregnancy appeared to increase the risk of GDM three-fold. These two biomarkers have previously been proposed as reliable markers for GDM screening (Anaka et al., 2014; Meek et al., 2016). Furthermore, in the lipid profile, low HDL-cholesterol in early pregnancy amongst the Saudi subjects independently proved to be a significant risk factor of GDM occurrence. This has similarly been reported previously in the UK and China (Savvidou et al., 2010; Makgoba et al., 2011; Li et al., 2015).

Vitamin D exerts a direct effect on pancreatic  $\beta$ -cells and glucose metabolism (Pittas & Dawson-Hughes, 2010). In the present study, vitamin D deficiency during early pregnancy was observed to be an independent risk factor for GDM, with a four-fold higher risk of development by mid-pregnancy. Blood levels of vitamin D were shown to exhibit an inverse correlation with HbA1c and fasting glucose in the present study. The insulin resistance observed at mid-pregnancy could possibly be due to vitamin D deficiency coinciding with the development of GDM. Similar findings have also been reported in a number of studies (Clifton-Bligh et al., 2008; Mutlu et al., 2015). Consistent with the findings of the present study, Lu and colleagues (2016) reported that vitamin D deficiency in early pregnancy increases the risk of GDM by 1.45 times. A number of meta-analyses have also confirmed that vitamin D deficiency in early pregnancy is a reliable predictor of GDM (Christesen et al., 2012; Poel et al., 2012; Aghajafari et al., 2013; Wei et al., 2013; Zhang et al., 2015; Lu et al., 2016).

Previous reviews, however, were based on observational studies and did not address all confounders. The majority of such studies were only adjusted for classical confounders, such as age, BMI, ethnicity, and family history (Maghbooli et al., 2008; Zhang et al., 2008; Arnold et al., 2015). In contrast, the current study investigated confounders such as physical activity, sun exposure, vitamin D intake, supplementation, season, socio-economic status, education, area of residence, GWG, history of diabetes, and parity. Vitamin D-related lifestyle factors were associated with a significant risk of developing GDM, such as sun exposure indices. In addition, multivitamin supplementation was found to confer protection against the development of GDM. Adjusting for sun

exposure and multivitamin intake was therefore important, because of its clinical relevance.

The current study revealed that obesity is associated with GDM, but not with vitamin D deficiency. It showed that as a confounder, obesity in early pregnancy did not attenuate the significance between vitamin D concentration and GDM risk. This is similar to Arnold et al. (2015), who revealed no statistical significance in the interaction of pre-pregnancy overweight/obesity status with 25(OH)D levels and GDM risk. These data imply that pregnant women, whether of normal weight or obese, need an early pregnancy vitamin D level of at least 50 nmol/L to reduce the risk of GDM, but this needs to be tested in future RCTs on supplementation.

GWG by mid-pregnancy showed a reduced weight-gaining trend among the GDM women in the current study, but GWG did not indicate any differences, with regard to vitamin D deficiency. These associations with GWG support previous studies on GDM (Hung & Hsieh, 2016) and vitamin D deficiency (Charatcharoenwitthaya et al., 2013; Bärebring et al., 2016). Vitamin D deficiency in early gestation increases the risk of GDM, and is independent of GWG as a confounder, while vitamin D deficiency at mid-pregnancy is found to be affected by GWG, and thus loses significance for GDM risk. To date, only one study has assessed GWG in relation to vitamin D levels at mid-pregnancy and GDM risk, and found that adjusting for BMI at mid-pregnancy contributes to the loss of significance for GDM risk, while GWG made little difference (Burris et al., 2012).

Multiparity, physical inactivity and low HDL-cholesterol were identified as independent risk factors of either vitamin D deficiency, GDM, or both. Adjusting for these confounders did not attenuate the significance of vitamin D increasing the risk of GDM here. Consistent with the findings of the present study, adjusting for physical activity has also been shown not to attenuate the risk posed by vitamin D deficiency for the development of GDM (Burris et al., 2012; Lacroix et al., 2014). Similarly, adjusting for parity here failed to attenuate the vitamin D-related risk of GDM, an observation

that has been reported previously (Maghbooli et al., 2008; Makgoba et al., 2011; Burris et al., 2012; Lacroix et al., 2014).

Despite the fact that 47.4% of the current subjects reported consuming multivitamins containing vitamin D, 36.7% were severely vitamin D-deficient by mid-pregnancy. This is because their supplementation failed to meet the 600 IU/day DRI. Daily supplementation with multivitamins and 400-500 IU of vitamin D has proved to be ineffective for the treatment of vitamin D deficiency (Lau et al., 2011). However one study has shown that in GDM women, the administration of 50,000 IU of vitamin D supplements twice in the study, along with 1000 mg calcium daily, effectively reduced fasting glucose, serum insulin levels, and HOMA-IR, and showed a significant improvement in lipid profile (Asemi et al., 2013). It has been proposed that the administration of 2000-4000 IU/day during pregnancy is sufficient for achieving adequate vitamin D levels (Hollis et al., 2011; Charatcharoenwitthaya et al., 2013; Wagner et al., 2013). Therefore, the administration of 5000 IU of vitamin D on a daily basis to women in early pregnancy brings about a significant reduction in future GDM development (Shahgheibi et al., 2016).

In the present study, vitamin D deficiency at mid-pregnancy was revealed to be an independent risk factor for metabolic syndrome, and this is an observation reported for the first time. It has been speculated that GDM could possibly be a component of metabolic syndrome, comprising hyperinsulinaemia, insulin resistance, obesity, dyslipidaemia, HTN, and T2DM, or impaired glucose tolerance (Ben-Haroush et al., 2004), meaning that vitamin D, GDM, and metabolic syndrome are interrelated.

## **7.2 Strengths of the Current Study**

The main strengths of the current study were:

1. The prospective study design and recruitment of a cohort from three tertiary care hospitals in Riyadh, using rigorous selection criteria. Strictly standardised

regimes for recruitment and data collection, facilitating individual follow-up for about 3-4 months.

2. All the women included in the study were ethnically homogeneous.
3. This is the first study in Saudi that links vitamin D deficiency in early pregnancy with GDM and metabolic syndrome.
4. For assessing GWG, the baseline weight was recorded during early pregnancy, as opposed to pre-pregnancy, for comparison with weight at mid-pregnancy.
5. For the first time ever, physical activity was documented as an independent risk factor for vitamin D deficiency during pregnancy, independent of sun exposure.
6. The use of an ECLIA permitted the detection of both forms of 25(OH)D. It was also highly correlated with the Liquid chromatography–mass spectrometry (LC-MS/MS) gold standard, and vitamin D results were included in the external quality assurance scheme, DEQAS (Zerwekh, 2008).

### **7.3 Limitations of the Current Study**

Some of the caveats have already been mentioned in previous chapters. However, the limitations of this study may be outlined as follows:

1. Significant high loss to follow-up.
2. Blood samples collected during early pregnancy were non-fasting specimens, whereas the samples collected mid-pregnancy were fasting samples. However, non-fasting samples were compared with fasting samples at the second visit, and fasting is not mandated for booking clinical visits during pregnancy. This is convenient for pregnant women, as in other studies (Savvidou et al., 2010; Tomedi et al., 2013; Lacroix et al., 2014). In fact, non-fasting TG in early pregnancy may be overestimated (Campos et al., 2005).
3. The FFQ used in the present study was designed to measure vitamin D, calcium, and protein intake, but not total energy, carbohydrates, or fats.
4. The recall bias of the questionnaires, including the FFQ, sun exposure questionnaire, and IPAQ, may have resulted in under- or over-reporting during



the interview. However, the interviewer was well-trained and used repeated questions to ensure the reliability of the answers.

5. Details of the compliance, dose, and type of multivitamin supplementation were not recorded.
6. Fat mass percentage was measured using skinfold thickness, which is vulnerable to error, especially during pregnancy (Forsum et al., 1989). However, for each patient, four sites were measured to minimise error. In addition, the most common and convenient method of measuring changes in body composition during pregnancy is anthropometry (skinfold thickness) (Forsum et al., 1989; Widen & Gallagher, 2014).
7. This study categorised GWG into excessive vs. non-excessive GWG. The non-excessive GWG included both adequate and inadequate GWG (i.e. below the IOM recommendations), similar to one recent meta-analysis (Brunner et al., 2015). Thus, the influence might be overestimated, and will partially result in potentially lowered GDM risk, associated with lower than recommended GWG.
8. This study used a HOMA equation to calculate insulin resistance and  $\beta$ -cell function. These are intended for low sensitivity and only reflect changes in the fasting state, compared to other direct methods, such as those derived from clamp studies or the minimal model. However, markers of insulin resistance/sensitivity based on fasting markers were strongly associated with gold-standard measures, and validated in the pregnant population (Kirwan et al., 2001).

## **7.4 Recommendations**

This thesis highlights the importance of studying low vitamin D concentration, prevalent among Saudi pregnant women living in Riyadh. More attention should be given to vitamin D concentration in pregnancy, due to its greater impact on health status and disease prevention than previously thought. Some strategies could be taken to address the current situation:

- Exposure to sunlight needs to be increased among women in the present context, through greater skin exposure across different parts of the body to the sun at noon, so that they can synthesise adequate levels of the vitamin.
- Awareness needs to be increased among pregnant women, with regards to the importance of dietary vitamin D intake from food naturally rich in vitamin D, or else food fortified with vitamin D.
- Enhanced fortification of food with vitamin D may be necessary to compensate for reduced vitamin D synthesis via the skin, and a reversal of vitamin D deficiency in Saudi Arabia, especially among pregnant women.
- Dietary supplementation of vitamin D, up to 2000-4000 IU/day should be considered for those at risk of vitamin D deficiency, such as pregnant women (Hollis et al., 2011). Furthermore, since a large proportion of pregnant women suffer from vitamin D deficiency, it would make sense to suggest vitamin D be given to all women planning a pregnancy or already pregnant, similar to folic acid.
- Indoor and outdoor physical activity is advised to help improve vitamin D status. An optimum duration of activity, amounting to a minimum of 30 minutes/day should be recommended.
- Effective education programmes should be designed to target Saudi pregnant women at the local level, in order to enhance public awareness of this serious problem; particularly among women of low socio-economic status. Thus, both personal and government action should be taken to address the current situation.

Understanding the high prevalence of GDM in Saudi women highlights the importance of investigating all the determinants and harnessing prevention strategies to reduce its incidence. Regarding GDM screening, random plasma glucose and HbA1c levels could be used to identify women at high risk of GDM, who would benefit from earlier diagnosis, or more intensive lifestyle intervention in early pregnancy.

A vital component in the prevention of both vitamin D deficiency and GDM may involve enhancing healthy maternal lifestyle factors, such as reducing weight before pregnancy, increasing physical activity, and restricting dietary cholesterol and calorie intake. It is in fact advisable for younger women to avoid fad foods high in fat and

sugar, as such intake may promote vitamin D deficiency and susceptibility to GDM, through an unbalanced diet. Furthermore, multivitamin and vitamin D supplementation represent a simple and safe intervention that could lower the risk of diabetes.

## **7.5 Future Directions**

- The significantly high prevalence of vitamin D deficiency among pregnant Saudi women indicates a need for large scale studies in KSA to assess vitamin D status, not only among pregnant women, but also among the general population.
- It is of paramount importance to assess the impact of lifestyle factors on vitamin D status among pregnant women, and the general population in the Saudi context, and this could take place by conducting large scale studies. It would include identifying the factors limiting sun exposure, with attempts being made to determine optimal levels of sun exposure for maintaining adequate levels of vitamin D among the local population.
- Although vitamin D status has been assessed in this study during early and mid-pregnancy, longitudinal studies are recommended for monitoring vitamin D status throughout pregnancy, as well as pregnancy outcomes among the local population.
- Further studies would clarify the interrelationship between sufficient vitamin D supplementation, recommended during pregnancy through RCTs in different trimesters, and would thus facilitate the establishment of guidelines for the Saudi population.
- Studies on inflammatory markers with vitamin D from early pregnancy and GDM risk are required. Both vitamin D and GDM are known to increase inflammation. Whether early pregnancy vitamin D deficiency and inflammation combined together increases risk of GDM needs to be explored.

## **7.6 Conclusion**

This study is among few Saudi studies that have measured vitamin D in early pregnancy, and is in fact the first Saudi study to assess the risk of vitamin D deficiency during early and mid-pregnancy, and investigating the effect of such deficiency on the prevalence of GDM. At 81% ( $< 50$  nmol/L), the prevalence of vitamin D deficiency

among pregnant Saudi women was found to be high, and poses a significant risk for the future development of GDM, which has reached 33%, according to IADPSG. Suboptimal sun exposure indices, multiparity, high total cholesterol-HDL ratio, low HDL-cholesterol, and living in West Riyadh were the main predictors of vitamin D deficiency among the pregnant Saudi women studied here. However, variables such as physical activity during pregnancy, and higher educational status seemed to confer some protection against vitamin D deficiency, whereas, the strongest risk factor for the development of GDM was a previous history of GDM. Furthermore, vitamin D deficiency in early pregnancy was found to increase the risk of GDM four-fold. Therefore, the monitoring of vitamin D status during pregnancy would appear to be a key factor in the early detection of vitamin deficiency, so timely therapeutic intervention could help avoid unfavourable pregnancy outcomes, complicated by GDM, particularly in the Saudi context.

## **APPENDICES**

## Appendix I- Ethical Approvals

Kingdom of Saudi Arabia  
Ministry of Higher Education  
**King Saud University**  
Code 034  
College of Medicine  
& King Khalid University Hospital

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



المملكة العربية السعودية  
وزارة التعليم العالي  
جامعة الملك سعود  
رمزها ٠٣٤  
كلية الطب  
ومستشفى الملك خالد الجامعي

Date: 11.02.2014 التاريخ: 11.04.1435 No.: 14/4067/IRB الرقم:

To : Dr. Abdulrahman Al Ajlan  
Riyadh College of Health Sciences

Re : Research Project No. E-13-1013  
"Prevalence of Vitamin D deficiency in pregnant women and its association with gestational diabetes mellitus"

Dear Dr. Al Ajlan,

Thank you for your response to the comments raised by the Board regarding the above-mentioned research project, which was reviewed and discussed in the IRB Meeting 4 (Academic Year 1434-1435) held on 26 December 2013 (23 Safar 1435). The IRB has reviewed your response and found that you have answered satisfactory and adequately fulfilled the requirements. Therefore, the project is now **approved**. Work on this project may begin.

We wish you success in your research and request you to keep the IRB informed about the progress of the study on a regular basis by submitting a *Study Progress Report* every 6 months and a *Final Report* when the study has been completed. If you make any changes to the protocol, you must submit a revised protocol to the IRB for approval before implementing the changes.

Thank you!

Sincerely yours,

**Dr. Khalid M. Al-Faleh**  
Chairman, Institutional Review Board  
King Saud University - College of Medicine  
Email: [kfaleh@ksu.edu.sa](mailto:kfaleh@ksu.edu.sa)

/rubie

IRB Registration Number with KACST, KSA: H-01-R-012  
IRB Registration Number with OHRP/NIH, USA: IRB00008644  
Approval Number Federal Wide Assurance NIH, USA: FWA00018774

April 21, 2012  
IRB Log Number: 12-081  
Category of Approval: **EXPEDITED**

Dear Dr. Aisha Mansoor Ali,

I am pleased to inform you that your study titled: 'Prevalence of Vitamin D Deficiency in Pregnant Women and its association with Gestational Diabetes Mellitus (GDM)' was reviewed and was approved.

Please be informed that in conducting this study, you as the Principal Investigator are required to abide by the rules and regulations of the Government of Saudi Arabia and KFMC/IRB. Further, you are required to submit a Progress Report before 21 March 2013; it can be reviewed by the IRB without lapse of approval. The approval of this proposal will automatically be suspended on 21 April 2012 pending the acceptance of the Progress Report. You also need to notify the IRB as soon as possible in the case of:

1. Any amendments to the project;
2. Termination of the study;
3. Any serious unexpected adverse events (within two working days).
4. Any event or new information that may affect the benefit/risk ratio of the proposal.

Please observe the following:

1. Personal identifying data should only be collected when necessary for research;
2. The data collected should only be used for this proposal;
3. Data should be stored securely so that a few authorized users are permitted access to the database;
4. Secondary disclosure of personal identifiable data is not allowed.
5. Copy of the Consent Form should be kept in the Research Subject's Medical Record and the consent process should be documented in the medical record.

الرفقات :

الرقم :

التاريخ :



Kingdom of Saudi Arabia  
Ministry of Health  
King Fahad Medical City



المملكة العربية السعودية  
وزارة الصحة  
مدينة الملك فهد الطبية

We wish you every success in your research endeavor.

If you have any further questions feel free to contact me.

Thank you.

*Sincerely, Yours,*

**Prof. Omar Hassan Kasule**  
Chairman - Institutional Review Board (IRB)  
King Fahd Medical City  
Riyadh, KSA  
Tel: + 966 1 288 9999 Ext. 7540  
E-mail: [okasule@kfmc.med.sa](mailto:okasule@kfmc.med.sa)





## **Appendix II -Consent form**

**Did the subject meet all study criteria while recruitment?**

Please check all the criteria

- Age from 18–39 years old
- First trimester between (8-12 weeks)
- Singleton pregnancy
- Saudi nationality
- Not on vitamin D supplementation
- No previous history of diabetes mellitus (type I or II).
- No calcium or parathyroid conditions or thyroid disease
- No history of usage cardiac medication therapy or diuretic.
- No chronic hypertension.
- No history of malabsorption.
- No serious chronic condition (epilepsy, cancer, other malignancy).

## إقرار بالموافقة على المشاركة في الدراسة

## Consent form

هذا مشروع مدعوم من قبل الخطة الوطنية للعلوم والتقنية برقم

12-MED2504-02

### Title of the study:

### عنوان الدراسة:

Prevalence of Vitamin D Deficiency in Pregnant Women and its Association with Gestational Diabetes Mellitus (GDM)

انتشار نقص فيتامين (د) لدى النساء الحوامل وارتباطه مع مرض سكر الحمل (GDM)

### Aim of the study:

### الهدف من الدراسة:

- To determine the prevalence of vitamin D deficiency in pregnant Saudi women
- To look for an association between vitamin D status and the incidence of gestational diabetes mellitus (GDM)
- To determine dietary vitamin D intake and calcium intake and associate it to the indices of obesity and GDM in Saudi pregnant women.
- To evaluate the influence of vitamin D status on foetal growth and development, as measured by foetus weight and fetal growth rate.

- ١. تحديد مدى انتشار نقص فيتامين (د) في النساء السعوديات الحوامل
- ٢. البحث عن وجود ارتباط بين حالة فيتامين (د) وحدوث مرض السكري الحمل (GDM).
- تحديد كمية فيتامين د والكالسيوم المتناوله وربطه بمؤشرات السمنة وداء السكري أثناء الحمل في النساء الحوامل السعوديه.
- تقييم تأثير حالة فيتامين D على نمو الجنين وتطوره بقياس وزن الجنين ومعدل نموه.

### I agree on the following:

### أقر أنا الموقع أثناءه:

- The doctor in charge has explained the study
- If I have questions or fears I can call the doctor or his assistant any time during the study.
- They can go through my medical records in relation to the study providing full confidentiality of my information.
- I know that participating in this study is by volunteer.
- Patient has the right to be withdrawn from the study at any time without mentioning reasons.
- Patient has the right in medical care and treatment
- I agree to participate in this study and I understood this agreement form and I will sign accordingly.

- أن الطبيب المسؤول شرح لي عن كيفية الدراسة
- إذا كانت لدي أي مخاوف أو أسئلة أخرى فيمكنني أستطيع الإتصال بالطبيب المعالج أو من ينوب عنه طوال فترة الدراسة.
- أوافق على أن من الممكن مراجعة سجلاتي الطبية ضمن إطار هذه الدراسة شريطة التأكد من أن هويتي الشخصية ستبقى سرية تماماً في جميع الأوقات.
- إنني أدرك أن الإشتراك طوعي في الدراسة.
- يحق للمشارك الانسحاب من الدراسة في أي وقت بدون ذكر الأسباب.
- يحق للمشارك الاهتمام من الرعاية الطبية والعلاج عند المخاطر.
- وأفهم تماماً نموذج الموافقة وما فيه من معلومات ، وبناءا على ذلك أوقعه طوعا وبملى إرادتي.

Participant name: .....

إسم المشترك : .....

Signature: .....

التوقيع : .....

Date: .....

التاريخ: .....

In charge doctor: .....

إسم الطبيب المسؤول : .....

Signature: .....

التوقيع: .....

Date: .....

التاريخ : .....

### Appendix III- First and Second visit questionnaires

First visit questionnaires		
Research code:	Date of filling of the form:	Date of filling of the form:
Name: Tel or Mobile No:	Place of birth: Week of gestation.....	Date of Birth (age):
<b>Anthropometric Measurements</b> <b><u>Mother anthropometrics :</u></b> ❖ Ht.....cm      Current Wt.....kg Pregnancy Wt..... kg Current BMI..... kg/ m2 Before pregnancy BMI..... kg/ m2  ❖ Waist:..... cm Hips:..... cm Waist-hips ratio:..... cm Mid arm Circumference..... cm  ❖ <b>Caliper:</b> Skin Fold thickness (fat %) .....  Triceps..... Biceps.....  Suprailiac..... Subscapular.....  ❖ <b>Blood Pressure:</b> .....		
<b>Sociodemographic Measurements</b>		
Education Level	<input type="checkbox"/> Illiterate <input type="checkbox"/> Primary <input type="checkbox"/> Intermediate <input type="checkbox"/> Secondary <input type="checkbox"/> University <input type="checkbox"/> Post graduate	
Family Income Per month	<input type="checkbox"/> No Income <input type="checkbox"/> less than 5000 S.R <input type="checkbox"/> 5000-10000 S.R <input type="checkbox"/> 10000-20000 S.R <input type="checkbox"/> More than 20000 S. R	
Occupation	<input type="checkbox"/> House wife <input type="checkbox"/> Retired <input type="checkbox"/> Student <input type="checkbox"/> Teacher <input type="checkbox"/> Employee <input type="checkbox"/> Physician <input type="checkbox"/> Businesswomen <input type="checkbox"/> Other (.....)	



**List of medications:****Please a check on all medications used by the subject*****Anti-Hyperlipidemics***

- ☐ Atorvastatin – Lipitor, Torvast  
☐ Cerivastatin – Lipobaby  
☐ Fluvastatin – Lescol, Lescol XL  
☐ Lovastatin – Mevacor, Altacor, Altoprev  
☐ Mevastatin ☐ Pitavastatin – Livalo, Pitava  
☐ Pravastatin – Pravachol, Selektine, Lipostat  
☐ Rosuvastatin – Crestor  
☐ Simvastatin – Zocor, Lipex  
☐ Simvastatin + Ezetimibe – Vytorin  
☐ Atorvastatin + Amlodipine – Caduet  
☐ Simvastatin + Niacin – Simcor  
☐ Cholestyramine ☐ Gemfibrozil  
☐ Colestipol ☐ Benzaifibrate, fenofibrate

***Cardiovascular drugs***

- ☐ Aspirin ☐ Warfarine ☐ Heparin  
☐ Clopidogrel ☐ Amiodarone –  
Amiodarone, Cordarone  
☐ Disopyramide – Rythmodan  
☐ Flecainide acetate – Apo – Flecainide

- ☐ Mexiletine – Novo – Mexiletine  
☐ Procainamide – Procan  
☐ Propafenone – Rhythrol, Nu, Apo, Gen, PMS  
☐ Digoxin  
☐ Clonidine  
☐ Methyldopa  
☐ Diazoxide  
☐ Hydralazine  
☐ Isosorbide dinitrate  
☐ Nitroglycerin  
☐ Prazosin, Terazosin, Dozazosin  
☐ Atenolol, Acebutolol, Bisoprolol, Labetalol,  
Metaoprolol, Nadolol, Oxprenolol, Pndolol,  
Propranolol, Sotalol, Timolol  
☐ Amlodipine, Felodipine, Nifedipine  
☐ Diltiazem, Verapamil  
☐ Captopril, Benazepril, Enalapril, Cilazapril,  
Perindopril, Quinapril, Ramipril, Lisinopril  
☐ Candesartan, Irbesartan, Losartan, Telmisartan,  
Valsartan,  
☐ Spironolactone ☐ Hydrochlorothiazide  
☐ Furosemide ☐ Pentoxifylline

***Other (Please specify)***

1. \_\_\_\_\_  
2. \_\_\_\_\_  
3. \_\_\_\_\_

Second visit questionnaires		
Research code:	Date of filling of the form:	Date of filling of the form:
Name: Tel or Mobile No:	Place of birth: Week of gestation.....	Date of Birth (age):
<b>Anthropometric Measurements</b> <b><u>Mother anthropometrics :</u></b> ❖ Ht.....cm      Current Wt.....kg Prepregnancy Wt..... kg Current BMI..... kg/ m2 Before pregnancy BMI..... kg/ m2  ❖ Waist:..... cm Hips:..... cm Waist-hips ratio:..... cm Mid arm Circumference..... cm  ❖ <b>Caliper:</b> Skin Fold thickness (fat %) .....  Triceps..... Biceps.....  Suprailiac..... Subscapular.....  ❖ <b>Blood Pressure:</b> .....		
<b>Clinical Measurements</b> ○ Pregnancy symptoms: <input type="checkbox"/> Nausea <input type="checkbox"/> vomiting <input type="checkbox"/> morning sickness <input type="checkbox"/> headache <input type="checkbox"/> mood swings <input type="checkbox"/> abdominal bloating <input type="checkbox"/> frequent urination <input type="checkbox"/> constipation <input type="checkbox"/> tender swallow breast		
○ Any complains or diagnosis.....  <input type="checkbox"/> Gestational HTN <input type="checkbox"/> Anemia <input type="checkbox"/> Viral or Bacterial illnesses <input type="checkbox"/> Preeclampsia <input type="checkbox"/> Respiratory <input type="checkbox"/> Gastrointestinal <input type="checkbox"/> bacterial vaginosis		

## First and Second visit

### Dietary intake of vitamin D and calcium Questionnaire

#### Breakfast cereals and bread

Daily				Weekly										Amount	
○Cornflakes	1	2	3	1	2	3	4	5	6	M	R	No	..... Cup/ week		
○Cornflakes with sugar and Coco Pops	1	2	3	1	2	3	4	5	6	M	R	No	..... Cup/ week		
○ Weciabix, Shredded wheat	1	2	3	1	2	3	4	5	6	M	R	No	..... Cup/ week		
○ Bran flakes, Wheat flakes or Sultana	1	2	3	1	2	3	4	5	6	M	R	No	..... Cup/ week		
○ Brown Bread	1	2	3	1	2	3	4	5	6	M	R	No	.....toast/ week or .....1/4 loaf		
○White Bread	1	2	3	1	2	3	4	5	6	M	R	No	.....toast/ week or .....1/4 loaf		
○White Bread(Samoli,burger)	1	2	3	1	2	3	4	5	6	M	R	No	.....toast/ week or .....1/4 loaf		
○Shaboora (bran or white)	1	2	3	1	2	3	4	5	6	M	R	No	.....pieces/week		

\*M (1-3 times per month), R(rarely), NO (don't take it at all)

#### Meat and Fish

Daily				Meat and Fish										Amount
White fish (hamoor), fish fingers	1	2	3	1	2	3	4	5	6	M	R	No	..... gm Fried / Grilled	
Oysters, shrimp	1	2	3	1	2	3	4	5	6	M	R	No	..... gm Fried / Grilled	
Canned fish (canned salmon, sardines,)	1	2	3	1	2	3	4	5	6	M	R	No	..... piece With oil / water	
Tuna fresh or canned	1	2	3	1	2	3	4	5	6	M	R	No	Can/large/small With oil / water	
Red meat (..... )	1	2	3	1	2	3	4	5	6	M	R	No	....gm/week With fat Little fat Without fat	
Chicken Type (..... )	1	2	3	1	2	3	4	5	6	M	R	No	....gm/week Skin/without skin	
OLiver, brain and kidneys	1	2	3	1	2	3	4	5	6	M	R	No	....gm/week With fat Little fat Without fat	

#### Egg

How many eggs do you eat?	1	2	3	1	2	3	4	5	6	M	R	No	.....Egg/ week
What kind of eggs do you eat? <input type="checkbox"/> yolk <input type="checkbox"/> White <input type="checkbox"/> Both													

#### Fats

What kind of fats used in your food?														<input type="checkbox"/> Ghee	<input type="checkbox"/> Margarine	<input type="checkbox"/> Butte	<input type="checkbox"/> Vegetables oil
Daily					Weekly								Amount				
○How many times do you use veg oil? Type....	1	2	3		1	2	3	4	5	6	M	R	No	.....Spoon/ week			
○How many times do you use olive oil?	1	2	3		1	2	3	4	5	6	M	R	No	.....Spoon/ week			
○How many times do you use butter or ghee? Type....	1	2	3		1	2	3	4	5	6	M	R	No	.....Spoon/ week			

○How many times do you eat Tahini? Type.....	1	2	3		1	2	3	4	5	6	M	R	No	.....Spoon/ week
○How many times do you usually have almonds or peanuts? Type.....	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week

\*Table spoon (15 ml), cup (240 ml)

Dairy And its Products														
Do you usually drink milk? <input type="checkbox"/> Yes <input type="checkbox"/> No														
If milk is liquid of which type? <input type="checkbox"/> Cow <input type="checkbox"/> Camel <input type="checkbox"/> Goat <input type="checkbox"/> Sheep <input type="checkbox"/> Other														
How many times do you drink fresh milk?														
Daily				Weekly										Amount
Full fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
Low fat	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
Skim fat	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
○ How many times do you drink dried milk?														
Daily				Weekly										Amount
Full fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
Low fat	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
Skim fat	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
○ How many time do you drink canned milk?														
Daily				Weekly										Amount
Full fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
Low fat	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
Full fat	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
Concentrated milk	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
Chocolate	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
With Strawberry	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
With Banana	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
other	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week
○How many times do you drink Laban?														
Daily				Weekly										Amount
Full fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
Low fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
Skim fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
How many times do you have yoghurt?														
Daily				Weekly										Amount
Full fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
Low fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
Skim fat	1	2	3		1	2	3	4	5	6	M	R	No	..... Cup/ week
○How many times do you have these products?														
Daily				Weekly										Amount
Triangle Cheese	1	2	3		1	2	3	4	5	6	M	R	No	.....Piece/ week



Kerry Cheese	1	2	3	1	2	3	4	5	6	M	R	No	.....Piece/ week
White Cheese	1	2	3	1	2	3	4	5	6	M	R	No	.....Spoon/ week
Yellow cheese	1	2	3	1	2	3	4	5	6	M	R	No	.....Spoon/ week
Fatty cheese	1	2	3	1	2	3	4	5	6	M	R	No	.....Spoon/ week
Geshtah	1	2	3	1	2	3	4	5	6	M	R	No	.....Spoon/ week
Labnah	1	2	3	1	2	3	4	5	6	M	R	No	.....Spoon/ week
Cream	1	2	3	1	2	3	4	5	6	M	R	No	.....Spoon/ week
Mozzarella	1	2	3	1	2	3	4	5	6	M	R	No	.....gm/ week
Tofu	1	2	3	1	2	3	4	5	6	M	R	No	.....Piece / week
* Food spoon= 15 gm													

Vegetables and Fruits													
○How many times do you eat the following?													
Daily				Weekly									Amount
Orange	1	2	3	1	2	3	4	5	6	M	R	No	.....One/ week
Fig	1	2	3	1	2	3	4	5	6	M	R	No	.....One/ week
Dried fruits (raisins,peache)	1	2	3	1	2	3	4	5	6	M	R	No	.....Cup / week
(fresh or cooked) kale	1	2	3	1	2	3	4	5	6	M	R	No	.....Cup / week
(fresh or cooked) celery	1	2	3	1	2	3	4	5	6	M	R	No	.....Cup / week
Spinach (fresh or cooked)	1	2	3	1	2	3	4	5	6	M	R	No	.....Cup / week
(fresh or cooked) broccoli	1	2	3	1	2	3	4	5	6	M	R	No	.....Cup / week
Mashroom (fresh or sundried)	1	2	3	1	2	3	4	5	6	M	R	No	.....Cup / week
Potatoes (fried or cooked)	1	2	3	1	2	3	4	5	6	M	R	No	.....One/ week
Homos,lentis	1	2	3	1	2	3	4	5	6	M	R	No	.....Cup / week
Fool (black beans)	1	2	3	1	2	3	4	5	6	M	R	No	.....Cup / week
*Cup = 240 m													
Cultural Food and others													
				Weekly									Amount
How many times do you have Greesh per week?				1	2	3	4	5	6	M	R	No	.....Cup/ week
How many times do you have Saleeq per week?				1	2	3	4	5	6	M	R	No	.....Cup/ week
How many times do you have Harees per week?				1	2	3	4	5	6	M	R	No	.....Cup/ week
How many times do you have Marase or msabeb per week?				1	2	3	4	5	6	M	R	No	.....Cup/ week
How many times do you have Pasta with cheese or milk (lasagna, fettuccine, bashamel) per week?				1	2	3	4	5	6	M	R	No	.....Cup/ week
How many times do you have pizza per week?				1	2	3	4	5	6	M	R	No	.....pieces/week
Sweets													
Daily				Weekly									Amount
How many times do you have Ice cream?	1	2	3	1	2	3	4	5	6	M	R	No	.....Piece/week Type.....
How many times do you have carbonated beverages?	1	2	3	1	2	3	4	5	6	M	R	No	.....Piece/week Type.....
How many times do you have chocolate?	1	2	3	1	2	3	4	5	6	M	R	No	.....Piece/week Type.....

How many times do you have muffins or cake or donuts?	1	2	3		1	2	3	4	5	6	M	R	No	.....Piece/week Type.....
How many times do you have biscuits?	1	2	3		1	2	3	4	5	6	M	R	No	.....Piece/week Type.....
How many times do you have cream caramel or custard?	1	2	3		1	2	3	4	5	6	M	R	No	.....Piece/week Type.....
<b>Tea and Coffee</b>														
<b>Daily</b>					<b>Weekly</b>									<b>Amount</b>
How many times do you drink red tea?	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week Type (.....)
How many times do you drink green tea?	1	2	3		1	2	3	4	5	6	M	R	No	.....Cup/ week Type (.....)
<b>Water</b>														
					<b>Daily</b>									<b>Amount</b>
How many times do you drink water?					1-3 cups		3-6 cups			6-12 cups			More than 12 cups	
Kind?			Bottled Water			water from Tanks			Tape water					

Supplements Intake	
Do you take Multivitamin? <input type="checkbox"/> Yes <input type="checkbox"/> No	Since when?.....  Do you take it regularly? <input type="checkbox"/> Yes <input type="checkbox"/> No How many times? <input type="checkbox"/> Daily <input type="checkbox"/> 3-4 times a week <input type="checkbox"/> once a week
Do you take Folic Acid? <input type="checkbox"/> Yes <input type="checkbox"/> No	Since when?.....  Do you take it regularly? <input type="checkbox"/> Yes <input type="checkbox"/> No How many times? <input type="checkbox"/> Daily <input type="checkbox"/> 3-4 times a week <input type="checkbox"/> once a week
Do you take Iron pills? <input type="checkbox"/> Yes <input type="checkbox"/> No	Since when?.....  Do you take it regularly? <input type="checkbox"/> Yes <input type="checkbox"/> No How many times? <input type="checkbox"/> Daily <input type="checkbox"/> 3-4 times a week <input type="checkbox"/> once a week
Do you take vitamin D supplement? If yes, which kind?? .....  <input type="checkbox"/> Yes <input type="checkbox"/> No	Since when?.....  Do you take it regularly? <input type="checkbox"/> Yes <input type="checkbox"/> No How many times? <input type="checkbox"/> Daily <input type="checkbox"/> 3-4 times a week <input type="checkbox"/> once a week  Amount.....IU or microgram
Do you take cod liver oil? <input type="checkbox"/> Yes <input type="checkbox"/> No	Since when?..... Do you take it regularly? <input type="checkbox"/> Yes <input type="checkbox"/> No How many times? <input type="checkbox"/> Daily <input type="checkbox"/> 3-4 times a week <input type="checkbox"/> once a week
Do you take any herbs? <input type="checkbox"/> Yes <input type="checkbox"/> No	First kind..... Since when? ..... Do you take it regularly? <input type="checkbox"/> Yes <input type="checkbox"/> No How many times? <input type="checkbox"/> Daily <input type="checkbox"/> 3-4 times a week <input type="checkbox"/> once a week  Amount.....
	Second kind..... Since when? ..... Do you take it regularly? <input type="checkbox"/> Yes <input type="checkbox"/> No How many times? <input type="checkbox"/> Daily <input type="checkbox"/> 3-4 times a week <input type="checkbox"/> once a week  Amount.....

Sun Exposure	
Current Season	<input type="checkbox"/> Summer <input type="checkbox"/> Winter
What is the extent of your exposure to the sun?	<input type="checkbox"/> No exposure <input type="checkbox"/> Daily Minutes (.....) <input type="checkbox"/> Weekly Minutes (.....)
What is the time of exposure to the sun?	<input type="checkbox"/> at sunrise <input type="checkbox"/> at noon <input type="checkbox"/> before sunset
What is the nature of your work?	<input type="checkbox"/> Outside/ directly under the sun <input type="checkbox"/> Inside (in covered rooms/ paces)
What are the parts of the body most exposed to the sun?	<input type="checkbox"/> Face <input type="checkbox"/> hands <input type="checkbox"/> Face and hands <input type="checkbox"/> Face, hands and feet
What is the extent of clothing cover to the body during exposure to the sun?	<input type="checkbox"/> Body except face <input type="checkbox"/> Body except face and hands <input type="checkbox"/> Body except face, hands and feet <input type="checkbox"/> The whole body <input type="checkbox"/> Body except hands and feet
Do you use sun protection creams?	<input type="checkbox"/> Yes (type.....) <input type="checkbox"/> No

<p><b>Physical activity (International Physical Activity Questionnaire) (IPAQ)</b></p> <p><b>READ:</b> I am going to ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.</p> <p><b>READ:</b> Now, think about all the <i>vigorous</i> activities which take <i>hard physical effort</i> that you did in the last 7 days. Vigorous activities make you breathe much harder than normal and may include heavy lifting, digging, aerobics, or fast bicycling. Think only about those physical activities that you did for at least 10 minutes at a time.</p> <p>1. During the last 7 days, on how many days did you do vigorous physical activities?</p> <p>_____ Days per week [VDAY; Range 0-7, 8,9]  8.Don't Know/Not Sure  9.Refused</p>
<p>2. How much time did you usually spend doing vigorous physical activities on one of those days?</p> <p>___ ___ Hours per day [VDHRS; Range: 0-16]  ___ ___ Minutes per day [VDMIN; Range: 0-960, 998, 999]  998.Don't Know/Not Sure  999.Refused</p>
<p>→Interviewer probe: An average time for one of the days on which you do vigorous activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "How much time in total would you spend over the last 7 days doing vigorous physical activities?"</p> <p>___ ___ Hours per week [VWHRS; Range: 0-112]  ___ ___ Minutes per week [VWMIN; Range: 0-6720, 9998, 9999]  9998.Don't Know/Not Sure  9999.Refused</p>
<p><b>READ:</b> Now think about activities which take <i>moderate physical effort</i> that you did in the last 7 days. Moderate physical activities make you breathe somewhat harder than normal and may include carrying light loads, bicycling at a regular pace, or doubles tennis. Do not include walking. Again, think about only those physical activities that you did for at least 10 minutes at a time.</p> <p>3. During the last 7 days, on how many days did you do moderate physical activities?</p> <p>_____ Days per week [MDAY; Range: 0-7, 8, 9]  8.Don't Know/Not Sure  9.Refused</p>

4. How much time did you usually spend doing moderate physical activities on one of those days?

\_\_ \_\_ Hours per day [MDHRS; Range: 0-16]  
\_\_ \_\_ Minutes per day [MDMIN; Range: 0-960, 998, 999]  
998. Don't Know/Not Sure  
999. Refused

➔[Interviewer probe: An average time for one of the days on which you do moderate activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, or includes time spent in multiple jobs, ask: "What is the total amount of time you spent over the last 7 days doing moderate physical activities?"

\_\_ \_\_ Hours per week [MWHRS; Range: 0-112]  
\_\_ \_\_ Minutes per week [MWMIN; Range: 0-6720, 9998, 9999]  
9998. Don't Know/Not Sure  
9999. Refused

**READ: Now think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.**

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

\_\_ \_\_ Days per week [WDAY; Range: 0-7, 8, 9]  
8. Don't Know/Not Sure  
9. Refused

6. How much time did you usually spend walking on one of those days?

\_\_ \_\_ Hours per day [WDHRS; Range: 0-16]  
\_\_ \_\_ Minutes per day [WDMIN; Range: 0-960, 998, 999]  
998. Don't Know/Not Sure  
999. Refused

➔[Interviewer probe: An average time for one of the days on which you walk is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "What is the total amount of time you spent walking over the last 7 days?"

\_\_ \_\_ Hours per week [WWHRS; Range: 0-112]  
\_\_ \_\_ Minutes per week [WWMIN; Range: 0-6720, 9998, 9999]  
9998. Don't Know/Not Sure  
9999. Refused

**READ: Now think about the time you spent sitting on week days during the last 7 days. Include time spent at work, at home, while doing course work, and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television.**

7. During the last 7 days, how much time did you usually spend *sitting* on a week day?

- \_\_ \_\_ Hours per weekday [SDHRS; 0-16]
- \_\_ \_\_ Minutes per weekday [SDMIN; Range: 0-960, 998, 999]
- 998.Don't Know/Not Sure
- 999.Refused

**➔[Interviewer probe]: An average time per day spent sitting is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "What is the total amount of time you spent *sitting* last Wednesday?"**

- \_\_ \_\_ Hours on Wednesday [SWHRS; Range 0-16]
- \_\_ \_\_ Minutes on Wednesday [SWMIN; Range: 0-960, 998, 999]
- 998.Don't Know/Not Sure
- 999.Refused

## Appendix IV -List of publications and abstracts

**Al-Musharaf, S.,** Al-Ajlan, A., Fouda, M., Turkistani, I., Aljohani, N., Al-Daghri, N. M., Tripathi, G., Saravanan, P. & McTernan, P. G. ( 2015) Vitamin D deficiency is associated with higher cardiometabolic risk in Saudi women during early pregnancy. *DIP Abstract.*

**Al-Musharaf, S.,** McTernan, P., Sarvanan, P., Al-Daghri, N., Al-Ajlan, A. & Sheshah, E. (2016) The negative impact of 25(OH)<sub>2</sub>D deficiency on sleep quality in Saudi women during their first trimester pregnancy. *Workshop of Vitamin D in Boston.*

**AL-Musharaf, S.,** McTernan, P., Sarvanan, P., Al-Daghri, N. & Al-Ajlan, A. (2016) 25(OH)<sub>2</sub>D deficiency and poor sleep quality during first trimester in pregnant Saudi women. *IOF Abstracts.*

AL-Ajlan, A., **AL-Musharaf, S.,** Krishnaswamy, S., Alokail, M.S., Aljohani, N.J., Al-Serehi, E., Alshingetti, N.M., Fouda, M.A., Turkistani, I.Z., Alharbi, A., Alraqebah, B.A., Ali, A.M., Al-Daghri, N.M. Lower Vitamin D Levels in Saudi Pregnant Women are Associated with Higher Risk of Developing GDM. Submitted to BMC Pregnancy and Childbirth.

Fouda, M.A., Turkistani, I.Z., **AL-Musharaf, S.,** AL-Ajlan, A., Angkaya–Bagayawa, F.F., Sabico, S., Mohammed, A.G., Hassanato, R., Al-Serehi, A., Alshingetti, N.M., Al-Daghri, N. M. Vitamin D Deficiency in Arab Pregnant Women. Submitted to Neonatology.



**Appendix V- Determinants of change in 25(OH)D between early and mid-pregnancy, for the whole cohort and deficient and non-deficient groups (Chapter 4)**

Parameters causing change in Vitamin D	Over all		Non-Deficient		Deficient	
	B	P-value	B	P-value	B	P-value
Pre-pregnancy BMI	-0.03± 0.08	0.703	-0.002± 0.005	0.718	0.001± 0.003	0.809
BMI at 1 <sup>st</sup> visit	0.00 ± 0.003	0.856	-0.001± 0.005	0.874	0.001± 0.003	0.641
BMI at 2 <sup>nd</sup> visit	0.00 ± 0.003	0.951	-0.001± 0.006	0.880	0.001± 0.003	0.733
GWG	-0.001 ± 0.004	0.723	0.005± 0.008	0.492	-0.002± 0.004	0.559
Multivitaminv at 1 <sup>st</sup> visit	-0.06 ± 0.05	0.158	-0.21±0.09	<b>0.021</b>	-0.03 ± 0.05	0.472
<b>Multivitaminv at 2<sup>nd</sup> visit</b>	0.07± 0.04	0.058	0.01 ± 0.08	0.915	<b>0.09 ± 0.04</b>	<b>0.025</b>
Low intensity PA (>1000mins/day) at 1 <sup>st</sup> visit	<b>-0.18 ± 0.05</b>	<b>0.000</b>	-0.05± 0.07	0.458	-0.06± 0.08	0.406
Low intensity PA (>1000mins/day) at 2 <sup>nd</sup> visit	<b>0.13 ± 0.05</b>	<b>0.005</b>	<b>0.16 ± 0.07</b>	<b>0.032</b>	<b>0.18± 0.05</b>	<b>0.000</b>
Sun Exposure at 1 <sup>st</sup> visit	0.05 ± 0.04	0.198	-0.006 ± 0.07	0.934	0.07 ± 0.04	0.075
Sun Exposure at 2 <sup>nd</sup> visit	0.01± 0.04	0.734	0.12 ± 0.08	0.144	-0.001 ± 0.04	0.983
Vitamin D intake at 1 <sup>st</sup> visit	-0.07 ± 0.12	0.569	0.16 ± 0.15	0.280	-0.10 ± 0.16	0.537
Vitamin D intake at 2 <sup>nd</sup> visit	0.19 ± 0.11	0.083	--	---	0.14 ± 0.10	0.175
Calcium intake at 1 <sup>st</sup> visit	-0.04 ± 0.08	0.590	0.17 ± 0.15	0.260	-0.08 ± 0.09	0.329
<b>Calcium intake at 2<sup>nd</sup> visit</b>	0.04 ± 0.06	0.445	<b>0.24± 0.11</b>	<b>0.031</b>	0.01± 0.06	0.863
Obesity at 1 <sup>st</sup> visit	0.03 ± 0.04	0.469	-0.04 ± 0.07	0.612	0.04 ± 0.04	0.311
Obesity at 2 <sup>nd</sup> visit	0.01 ± 0.03	0.667	0.03 ± 0.07	0.678	0.01 ± 0.04	0.740
Season (summer) at 1 <sup>st</sup> visit	-0.02 ± 0.03	0.547	0.03 ± 0.07	0.718	-0.02 ± 0.04	0.542
Season (summer) at 2 <sup>nd</sup> visit	-0.03 ± 0.03	0.381	-0.08 ± 0.07	0.217	-0.01 ± 0.04	0.734
Body Fat (%) at 1 <sup>st</sup> visit	0.00 ± 0.003	0.889	-0.002 ± 0.006	0.739	0.004 ± 0.003	0.208
Body Fat (%) at 2 <sup>nd</sup> visit	0.00 ± 0.005	0.964	-0.01 ± 0.01	0.207	0.01 ± 0.01	0.226
HbA1c at 1 <sup>st</sup> visit	0.04 ± 0.04	0.212	-0.05 ± 0.06	0.458	0.04 ± 0.04	0.296
<b>HbA1c at 2<sup>nd</sup> visit</b>	-0.07 ± 0.04	<b>0.065</b>	0.02 ± 0.08	0.832	<b>-0.11 ± 0.04</b>	<b>0.005</b>
Sqrt HOMA-B	0.001 ± 0.001	0.637	0.0 ± 0.004	0.975	0.00 ± 0.001	0.436
Sqrt HOMA-IR	-0.02 ± 0.02	0.329	-0.04 ± 0.06	0.500	-0.02 ± 0.02	0.303
Metabolic syndrome	0.03 ± 0.04	0.525	0.03 ± 0.10	0.782	0.01 ± 0.05	0.824
<b>TG (≥ 1.7 mmol/l) at 1<sup>st</sup> visit</b>	-0.04 ± 0.04	0.213	<b>-0.17 ± 0.07</b>	<b>0.021</b>	-0.03 ± 0.04	0.414
TG (≥ 1.7 mmol/l) at 2 <sup>nd</sup> visit	0.05 ± 0.04	0.188	-0.05 ± 0.07	0.509	0.05 ± 0.04	0.160
Low HDL-cholesterol (< 1.03 mmol/l) at 1 <sup>st</sup> visit	0.02 ± 0.03	0.585	0.07 ± 0.07	0.325	-0.02 ± 0.04	0.608
Low HDL-cholesterol (< 1.03 mmol/l) at 2 <sup>nd</sup> visit	-0.04 ± 0.04	0.220	-0.06 ± 0.08	0.485	-0.07 ± 0.04	0.057

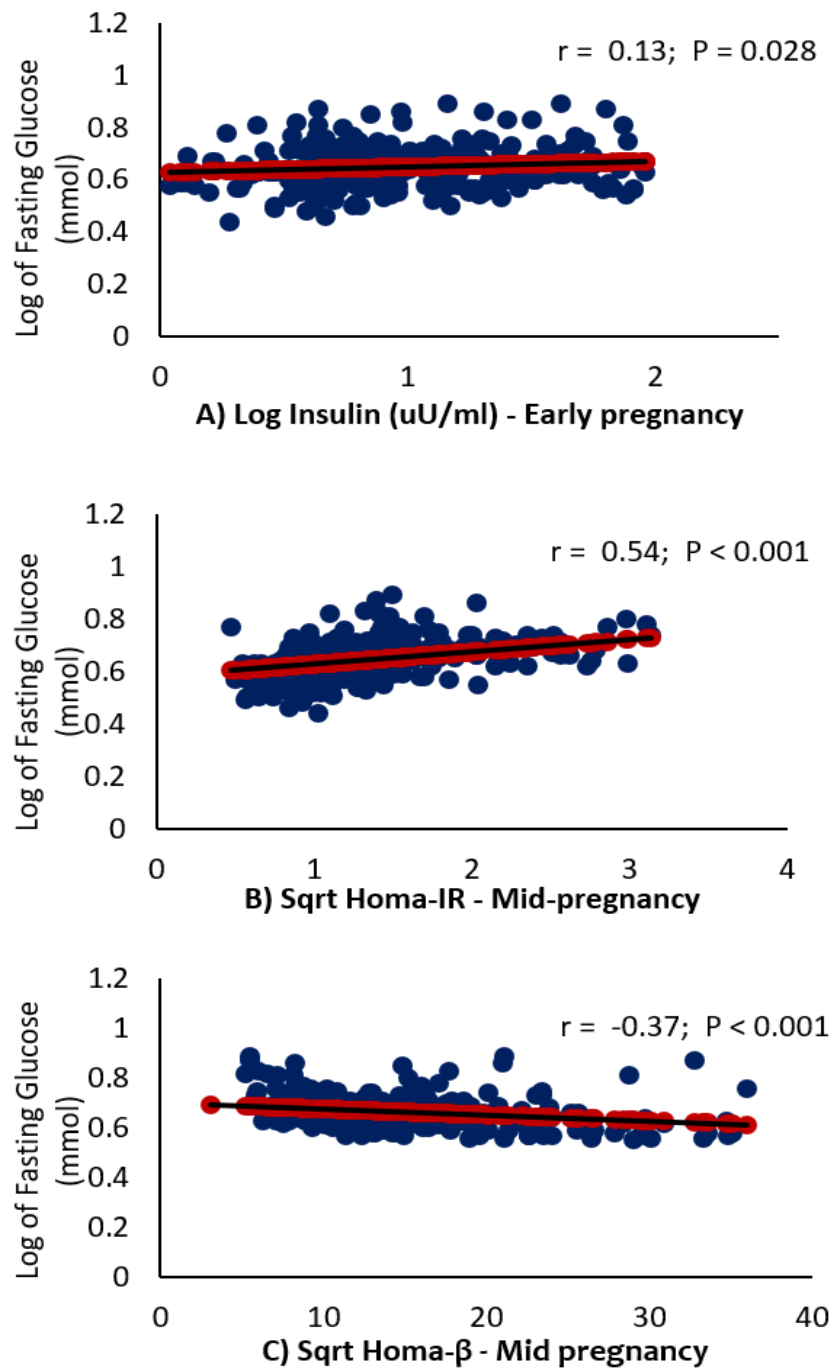
**Note:** the blank is because no patients in the non-deficient group took vitamin D (>600 IU/day). The change of vitamin D between visits is calculated by vitamin D at visit 2 – vitamin D at visit 1. Positive sign shows it helps increase the level of Vitamin D at mid-pregnancy.

**Appendix VI- Gestational weight gain according to BMI in both visits (Chapter 5)**

		Total Gestational weight gain					P-values
		Mean	SD	Median	Q1	Q3	
Pre- pregnancy BMI	underweight	9.2	3.9	8.6	6	11.55	0.001
	Normal	7.9	3.8	7.85	5.3	9.5	
	Overweight	7.2	4.1	7.1	4.9	9.05	
	Obese	5.7	4.8	5.35 <sup>ABC</sup>	3.65	7.6	
BMI at 1 <sup>st</sup> visit	underweight	8.0	4.3	6.5	5.2	9.6	0.001
	Normal	8.2	3.8	8.1	5.55	9.95	
	Overweight	7.4	3.8	7	4.9	9	
	Obese	5.9	4.9	5.6 <sup>B</sup>	4	8	
BMI at 2 <sup>nd</sup> visit	underweight	6.8	2.5	5.5	5.2	9.6	0.583
	Normal	7.1	3.6	7.4	4.6	9	
	Overweight	7.6	3.5	7.35	4.9	9	
	Obese	6.9	5.1	6.4	4.5	9.3	

**Note:** superscripts ABC indicates significance from Underweight Normal and Overweight respectively.

## Appendix VII- Correlations (Chapter 5)



**Figure** Correlation between Log fasting glucose (mmol/l) with sqrt **A)** Log Insulin (uU/ml) at early pregnancy and **B)** sqrt HOMA-IR and **C)** sqrt HOMA- $\beta$  at mid-pregnancy.

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